AN INVESTIGATION INTO THE MECHANISMS INVOLVED IN THE DEPRESSION OF OVULATION RATES IN EWES GRAZING OESTROGENIC LUCERNE

A thesis presented in partial fulfilment of the requirements for the degree of Master of Agriculture Science in Animal Science at Massey University

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A B S T R A C T

The reproductive performance of 123 Romney and Border Leicester x Romney ewes was compared after they grazed oestrogenic lucerne or non-oestrogenic ryegrass/clover pasture.

To synchronize oestrus prior to the experimental treatment, progestagen-impregnated intravaginal sponges were inserted in all ewes for 14 days. In Experiment I, which involved 42 Romney and Border Leicester x Romney ewes, the animals grazed treatment pastures for one complete oestrous cycle. Blood samples were taken at intervals throughout the cycle and luteinizing hormone concentrations were determined. Ovulation rates and the numbers of follicles present on the surface of the ovaries were recorded at laparotomy, three days post-oestrus.

In Experiment II, which involved 81 Romney ewes, oestrogenic lucerne or non-oestrogenic ryegrass/clover pasture was grazed for a complete oestrous cycle, or treatments were interchanged in mid-cycle. All ewes were slaughtered three days post-oestrus and their reproductive tracts were recovered and individually identified. Ovulation rates and the numbers of follicles on the surface of the ovaries were recorded. After sectioning the ovaries, all follicles of a diameter greater than 2.0mm were recorded. Sections of tissue from the vagina, cervix, uterus and fallopian tubes of each ewe were mounted, stained with haematoxylin and eosin, and the height of their epithelial cells measured.

The ingestion of oestrogenic lucerne for one complete oestrous cycle depressed ovulation rates by 29 percent (0.67 ovulations per ewe) in Experiment I and by 22 percent (0.40 ovulations per ewe) in Experiment II. The ingestion of oestrogenic lucerne for part of the oestrous cycle,

depressed ovulation rates only if it was consumed for the latter half of the cycle.

Oestrogenic lucerne did not significantly influence the secretion of luteinizing hormone over the pre-ovulatory period, or at any other stage of the oestrous cycle.

There were no significant differences in the total numbers of ovarian follicles present, in the numbers of follicles on the surface of the ovaries, or in the numbers of large follicles (with a diameter of greater than 3.5mm) present, between the treatment groups.

The ingestion of oestrogenic lucerne did not increase significantly, the height of epithelial cells in genital tract tissue, when compared with that in ewes grazing non-oestrogenic ryegrass/clover pasture. Similarly, there was no significant difference in uterine weights. This is in contrast to published data where lucerne is fed to speyed ewes.

A C K N O W L E D G E M E N T S

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PREFACE

This investigation was conducted at the Fertility Centre, part of the Department of Sheep Husbandry, Massey University. The experimental work was carried out in April and May of 1977 and represents original research by the author under the supervision of Dr. M.F. McDonald, Reader, Sheep Husbandry Department, Massey University.

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INTRODUCTION

Lucerne (*medicago sativa*) has become increasingly important as a forage crop in those parts of New Zealand which experience dry summer conditions. Its ability to produce high yields of forage, of high nutritive value, whether grazed *in situ* or fed as hay or silage, has led to its popularity. In these areas lucerne production frequently exceeds that of conventional pastures.

Because it produces forage of high nutritive value in late summer and autumn, lucerne would appear to be ideal for 'flushing' ewes prior to mating. However, lucerne has been shown to contain oestrogenic compounds which impair the reproductive performance of ewes. The phyto-oestrogens, the coumestans, and to a lesser degree the isoflavones, may reach high concentrations in lucerne leaf material, and the coumestans have been shown to lower lambing percentages. This depression in lambing percentage is related to a reduction in ovulation rates.

An understanding of those mechanisms involved in the lucerne - induced depression of ovulation rates would be of considerable advantage. It may enable management practices to be devised, that would allow lucerne to be utilized in achieving optimum ewe liveweights prior to tupping, without incurring any loss in reproductive performance.

C H A P T E R I

REVIEW OF LITERATURE

C H A P T E R I

REVIEW OF LITERATURE

(A) PHYTO-OESTROGENS

The term 'oestrogen' originally referred to certain steroid hormones secreted by the ovary. The development of the Allen-Doisy vaginal smear test in 1923 provided a wider definition of oestrogenicity. 'An oestrogen is any pure chemical substance which, when injected into adult ovariectomized mice or rats, produces cornification of the vagina, similar to that occurring during oestrus in the normal intact animal' (Allen and Doisy, 1923).

The occurrence of oestrogenic substances in plants was first reported in 1926 (Dorhn et al., 1926; Fellner, 1926; Loewe, et al., 1927) and the first oestrogen to be isolated and identified in the plant world was oestrone, extracted from the oil palm (Butenandt and Jacobi, 1933). Consequently several compounds closely resembling the natural steroid oestrogens were isolated from a number of plants. An oestriol-like compound was extracted from willow catkins (Skarzynski, 1933), and from liquorice root (Costello and Lynn, 1950). A creeper claimed by the Chinese to possess aphrodisiac properties was shown to contain miro-oestrol (Jones and Pope, 1961) and plants used for contraceptive purposes in India and China contained psoralidin (Dattagupta et al., 1960).

(i) Phyto-Oestrogens in Pasture Plants

When Bennetts, Underwood and Shier (1946) demonstrated an infertility syndrome affecting ewes grazing subterranean clover pasture (Trifolium subterraneum L var. Dwalganup), they provided strong presumptive evidence of the presence of an oestrogen. This was confirmed by Curnow et al., (1948) who showed the oestrogenic activity of ether extracts of subterranean clover; and supported indirectly by East et al., (1949) with the demonstration of a protective action of androgen injections to

sheep grazing clover pastures. Oestrogenic activity was also attributed to red clover (Pope, 1954), lucerne (Coop and Clark, 1960) and red clover and lucerne hay (Cheng et al., 1953).

In 1951 Bradbury and White succeeded in isolating two isoflavones from subterranean clover extract; 7-hydroxy-4'-methoxy isoflavone (formononetin) and 5:7:4'-trihydroxy isoflavone (genistein). Genistein was also isolated from red clover (Pope and Wright, 1954; Curnow and Rossiter, 1955; Wong, 1962), and from white clover and lucerne (Guggolz et al., 1961). Subsequently the isoflavones 7:4'-dihydroxy isoflavone (diadzein), 5:7-dihydroxy-4'-methoxy isoflavone (biochanin A), and 5:7:3'-trihydroxy-4'-methoxy isoflavone were isolated from both subterranean and red clovers (Livingstone et al., 1961). These isoflavones were all shown to be oestrogenic (Bradbury and White, 1954; Moule et al., 1963), and their synthetic analogues were shown to be of equal oestrogenic potency (Cheng et al., 1954).

Bickoff et al., (1957; 1958) isolated a crystalline substance of oestrogenic nature from ladino clover, strawberry clover and alfalfa (lucerne). This compound was not an isoflavone but a benzofurocoumarin derivative - a class later named 'coumestans'. Bickoff proposed the name 'coumestrol' for this oestrogenic compound which proved to be 30 times more potent than genistein (Bickoff et al., 1958). Coumestrol and the non-oestrogenic 4'-methoxycoumestrol, have since been detected in red clover, subterranean clover and burr clover (Lyman et al., 1959). In addition, six other coumestans have been isolated from lucerne (Bickoff et al., 1964) and from ladino clover (Bickoff et al., 1965). The coumestans were later shown to be biogenetically related to the isoflavones (Grisebach and Barz, 1963).

Until recently the most commonly used method of measuring the oestrogenic potency of a phyto-oestrogen has been the mouse uterine

response. However difficulties have often arisen in attempting to correlate results obtained with mice and other laboratory animals, to the results obtained under field conditions with farm animals (Morley et al., 1968). Bickoff and co-workers (1962) compared the oestrogenic potencies of orally fed phyto-oestrogens and steroid oestrogens, with injected administration in mice. Braden et al., (1967) made similar comparisons using ovariectomized ewes. The comparative potencies are shown in Table 1-1.

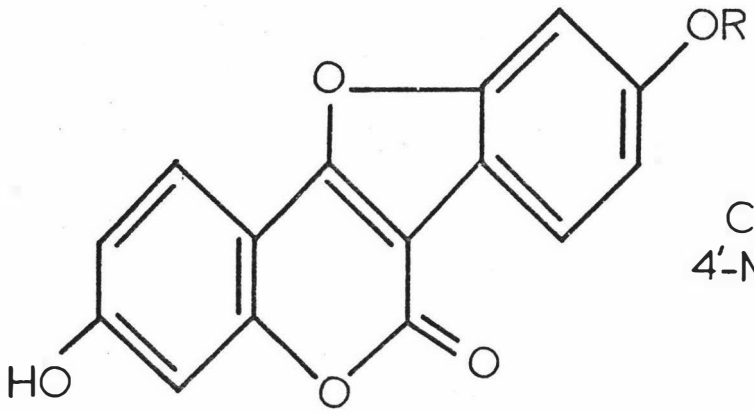
TABLE 1-1: THE RELATIVE POTENCIES OF PHYTO-OESTROGENS ADMINISTERED ORALLY OR INTRAMUSCULARLY

OESTROGEN	MOUSE		EWE	
	ORAL	I/M	ORAL	I/M
Diethylstilboestrol	100,000	100,000	100,000	100,000
Oestrone	6,900	6,900	6,900	6,900
Coumestrol	35	35	9	35
Genistein	1.5	1.5	0.07	1.5
Diadzein	0.4	0.4	0.02	0.4
Biochanin A	1.0	1.0	0.05	1.0
Formononetin	negligible	negligible	0.06	negligible

from Bickoff et al., (1962), Braden, et al., (1967) and Wong and Flux, (1962)

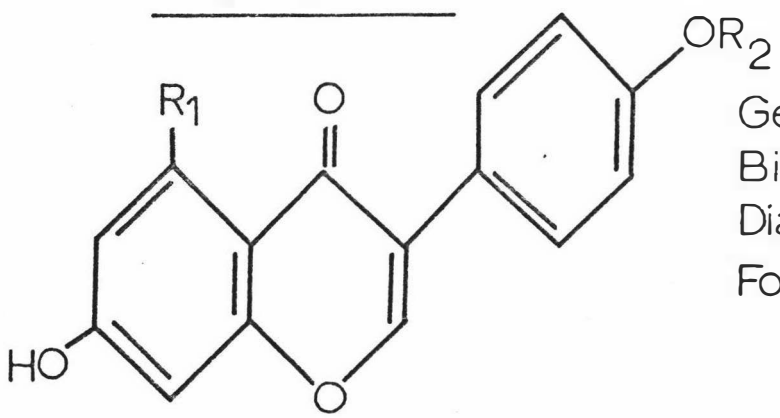
Despite the relatively low potencies of the phyto-oestrogens, they are present in certain forages in sufficient quantities to influence animal physiology. The structures of the phyto-oestrogens are shown in FIG 1-1.

Fig. 1·1 PHYTO-OESTROGENS



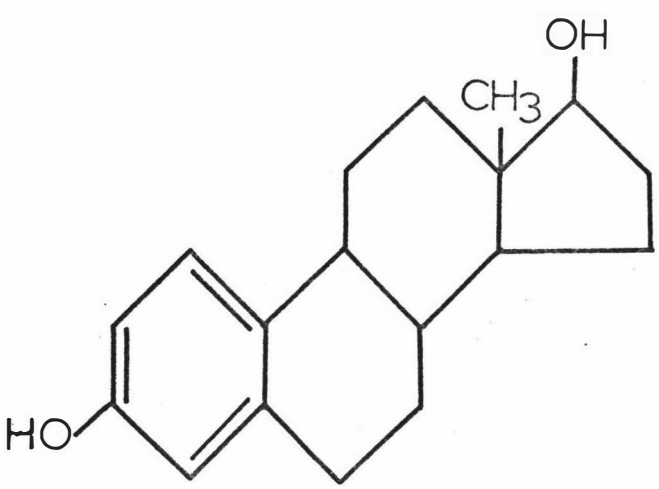
Coumestrol : R = H
4'-Methoxy coumestrol: R = CH_3

COUMESTANS



Genistein : R_1 = OH R_2 = H
Biochanin A : R_1 = OH R_2 = CH_3
Diadzein : R_1 = H R_2 = H
Formononetin : R_1 = H R_2 = CH_3

ISOFLAVONES



OESTRADIOL-17β

(ii) Factors Influencing Phyto-oestrogen Levels

Investigations have shown that there is considerable variation in the oestrogenic potency of pasture plants and that this is influenced by both environmental and genetic factors.

GENETIC: Bradbury and White (1954) tabulated over 50 different species of plants reported as oestrogenic. There is variation between species in the ratio of coumestans to isoflavones. The coumestans are the dominant source of oestrogenicity in lucerne (Bickoff et al., 1962), ladino clover (Guggolz et al., 1961), the annual medics (Bennett et al., 1967b; Francis and Millington, 1965a; Millington et al., 1964a) and white clover (Wong et al., 1971). The isoflavones account for most of the oestrogenicity of subterranean clover (Millington et al., 1964b) and red clover (Wong, 1962).

Wide variations in oestrogen levels are also evident between varieties and between lines within a species; in lucerne (Francis and Millington, 1965a; Stob et al., 1957; Stuthman et al., 1967), subterranean clover (Francis and Millington, 1964; Davies and Dudzinski, 1965) and red clover (Francis et al., 1967). Francis and Millington 1965b) treated subterranean clover seed with a mutagenic agent and produced a number of mutant plants of very low oestrogenicity.

ENVIRONMENTAL: Nutrient deficiencies influence the oestrogen content of pasture plants. Oestrogenic activity has been shown to increase significantly when phosphate deficiencies occur in subterranean clover (Alexander and Rossiter, 1952) and in red clover (Rossiter and Beck, 1966b; Butler et al., 1967). Concentrations of formononetin were most affected although diadzein and biochanin A levels also increased (Rossiter and Beck, 1966b). Severe deficiencies in sulphur (Rossiter, 1970; Rossiter and Barrow, 1972), and in nitrogen (Rossiter, 1969) can almost double formononetin levels.

Pieterse and Andrews (1956) and Kohler and Bickoff (1961) found that coumestrol levels increased proportionally with maturation of lucerne

plants. Francis and Millington (1965a) demonstrated a similar trend in annual medics.

Oestrogenicity is reported to fall dramatically after the harvesting and drying of subterranean clover (Francis and Millington, 1965c), and of lucerne (Bickoff et al., 1959; 1960a; Livingstone et al., 1961). Commercial dehydration has a similar effect (Francis and Millington, 1965a). In contrast, lucerne silage is more potent than the green lucerne from which it is produced (Pieterse and Andrews, 1956).

There are conflicting reports on the effects of high temperatures (Coleman et al., 1965; Loper and Hanson 1964; Rossiter and Beck, 1966a), season (McLean, 1967; Squires, 1966) and location (Millington et al., 1964a), on oestrogen levels of pasture plants.

Bickoff et al., (1960b) noted that a disease-infected sample of white clover contained considerably more oestrogenic activity than comparable disease-free samples. Subsequently, a strong correlation has been reported between pathogenic attack of lucerne and of white clover, and coumestrol levels (Bennett et al., 1967a; Wong and Latch, 1971). Two leaf-spotting organisms shown to be implicated are Leptosphaerulina briosiana (Loper and Hanson, 1964), the fungal organism of 'Pepper spot' disease; and Pseudopeziza medicaginis (Loper and Hanson, 1964), the causal organism of 'common leafspot'. Loper et al., (1967) found that coumestrol content was very high in the necrotic lesions of infected leaves, and that selection of plants for disease resistance, or the use of fungicidal agents, markedly reduced coumestrol levels. Infestation of lucerne by aphids has also been shown to cause a build-up of coumestans (Hanson et al., 1965; Loper, 1968).

The accumulation of coumestrol following pathogenic attack may be implicated in some non-specific disease resistance mechanism. There is considerable data showing that phenolic compounds are formed in plants subjected to pathogenic attack or physical damage (Farkas and Kiraly, 1962). The 'Phytoalexin Theory' (Ingham, 1972) proposes that a group of phenolics

the phyto-alexins - which exhibit antifungal properties, are produced by plant cells as a result of pathogenic attack. Coumestans appear not to have antifungal properties, but they are structurally similar to phyto-alexins. It is suggested that both these compounds arise from a common biosynthetic pathway (Ingham, 1972).

In summary, it can be stated that although several environmental factors may contribute to wide variation in the oestrogenicity of pasture plants, the presence of pathogenic disease is the single most influencing factor.

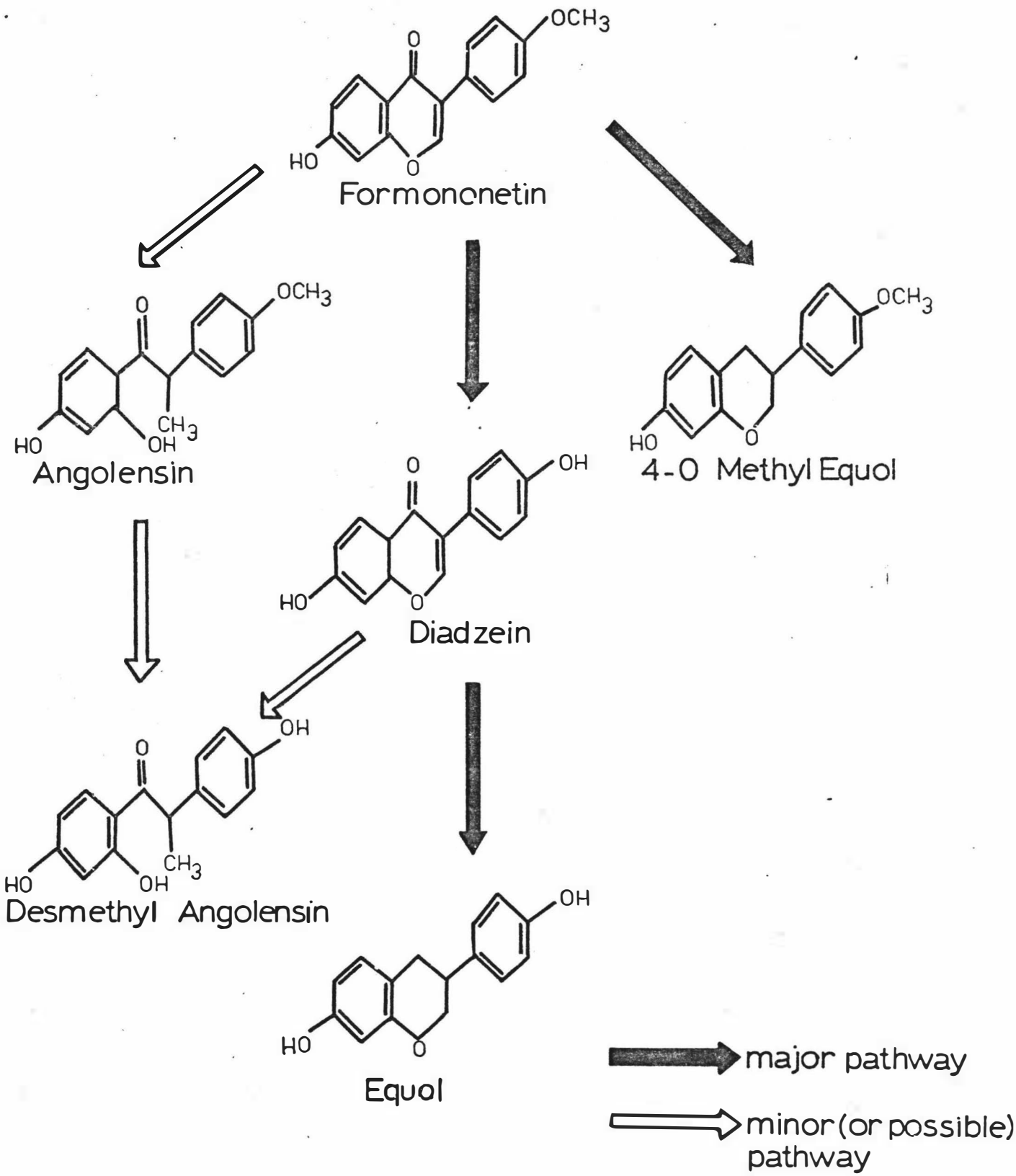
(iii) The Metabolism of Phyto-oestrogens in Sheep.

When considering the biological effects of phyto-oestrogens, of particular importance are the metabolic changes that they undergo within the animal.

The presence of a microsomal enzyme system in a number of animal species, including sheep, which catalyses the demethylation of isoflavones has been reported (Nilsson, 1963). It was demonstrated that the major reaction occurring to biochanin A and formononetin incubated in sheep rumen liquor, is demethylation to genistein and diadzein respectively (Nilsson, 1961). There was no evidence to suggest more extensive metabolism *in vivo* than was demonstrated *in vitro* (Batterham *et al.*, (1965). Subsequent investigations using radioactive isotopes (^{14}C) confirmed that the products of metabolism of formononetin were diadzein and 7:4'-dihydroxy isoflavone (equol) (Nilsson *et al.*, 1967; Batterham *et al.*, 1971). About 70 percent of the formononetin ingested by sheep is converted to urinary equol, together with a small amount of diadzein, desmethylangolensin (Batterham *et al.*, 1971), O-methyl equol and angolensim (Cox and Braden, 1974). The pathways of formononetin metabolism in sheep are shown in Fig. 1-2.

Formononetin is of negligible oestrogenic potency if administered to sheep via intramuscular or intravenous pathways, whereas a much greater oestrogenic activity is apparent if administered intra-ruminally

Fig. 1·2 THE METABOLISM OF FORMONONETIN IN SHEEP



Braden et al., 1967). In contrast, equol proves to be oestrogenically active when administered intramuscularly (Shutt and Braden, 1968). Furthermore high levels of equol are excreted in the urine of sheep grazing oestrogenic clovers (Shutt et al., 1967; 1970). Equol is the predominant phyto-oestrogen in digesta and excretia of sheep grazing clovers with high formononetin content (Shutt et al., 1970). Formononetin consequently is now considered to be the most important isoflavone in terms of oestrogenic activity, although it has little oestrogenic activity itself. Differences in oestrogenicity between strains of subterranean clover have been related to their formononetin content (Lindsay and Francis, 1968; Davies et al., 1970). O-methyl equol and desmethyl angolensin are somewhat less oestrogenic than equol (Micheli et al., 1962).

Little is known of the metabolism of coumestans in sheep. The demethylation of 4'-methoxy coumestrol to coumestrol has been demonstrated in vivo (Shutt et al., 1969) and in vitro (Adler and Weitzkin-Neiman, 1970).

Braden et al., (1967) were unable to find any obvious metabolic products in the urine or plasma of sheep given coumestrol intra-ruminally. Cayen and Common (1965), feeding tritiated coumestrol to chickens, were unable to detect any radioactive urinary equol, suggesting different metabolic pathways for the coumestans than for the isoflavones.

Less than 1 percent of the daily intake of phyto-oestrogens are excreted as such in the faeces or urine (Shutt et al., 1970). The phyto-oestrogens and their metabolites are absorbed, largely from the rumen, and circulate in the blood. Plasma of sheep grazing oestrogenic clover contains water-soluble conjugates of diadzein, genistein and biochanin A - mainly in the form of glucuronides (Shutt et al., 1967; Lindner, 1967; Beck and Knox, 1971).

Plasma from sheep fed synthetic coumestrol or medic hay, contains

water-soluble conjugates of coumestrol and free coumestrol, but 4-methoxy coumestrol is not present (Shutt et al., 1969).

Levels of conjugated phyto-oestrogens, both isoflavones and coumestans, are considerably higher than levels of free phyto-oestrogens (Wong and Cox, 1972). As well as conjugates of glucosiduronates, relatively high concentrations of sulpho-conjugates have been isolated from sheep plasma (Wong and Cox, 1971).

(iv) Phyto-oestrogens and Reproductive Failure in Sheep

(a) Isoflavones

CLOVER DISEASE - The classical infertility syndrome associated with ewes exposed for prolonged periods to oestrogenic subterranean clover pastures, is a permanent depression in fertility. This infertility syndrome, which became known as 'clover disease', was first reported by Bennetts, Underwood and Shier in 1946. They described dramatic decreases in lambing percentages (by up to 90%), a high incidence of dystocia, uterine prolapse months after parturition or even in unbred ewes, and copious milk secretion in virgin and non-pregnant ewes (Bennetts et al., 1946; Bennet, 1966). In wethers, enlargement of the teats and induction of lactation is common; and in ewes, the pathological condition, cystic glandular hyperplasia, is almost always found in the cervix and uterus, (Bennetts et al., 1946; Barrett et al., 1961). 'Clover disease' has a high degree of permanence, persisting for periods of several years, if not for the lifetime of the ewe, after the ewes are removed from oestrogenic pastures (Schinckel, 1948; Underwood and Shier, 1951; Barrett, et al., 1965; Turnbull et al., 1966).

Subsequent investigations have indicated that the main cause of infertility is the failure of ova to be fertilized (Turnbull et al., 1966; Lightfoot et al., 1967; Kaltenbach and Davies, 1970; Lightfoot et al., 1974; Wroth and Lightfoot, 1976). The establishment of a reservoir of spermatozoa in the cervix immediately after mating, is necessary for

normal fertility in sheep (Mattner, 1966), and sperm movement within the tract of 'clover disease' effected ewes is impaired. The numbers of sperm within the cervix (Turnbull et al., 1966; Lightfoot et al., 1967; Kaltenbach and Davies, 1970; Lightfoot et al., 1974), within the fallopian tubes (Turnbull et al., 1966; Lightfoot et al., 1967; Kaltenbach and Davies, 1970), and adhering to the zona pellucida of recovered ova (Lightfoot et al., 1974; Wroth and Lightfoot, 1976), are much lower than in 'normal' animals.

The rheological properties of cervical mucus are important in the establishment and retention of a population of sperm in the cervix of the ewe (Gibbons and Mattner, 1966), and the visco-elasticity of cervical mucus is decreased in ewes affected by 'clover disease' (Smith, 1971; Adams, 1976a). Adams (1977) showed that spinbarkheit of cervical mucus could be related to lowered reproductive performance of ewes exposed to oestrogenic pasture.

Evidence for a higher than normal incidence of embryonic mortality (Turnbull et al., 1966; Kaltenbach and Davies, 1970),diminished ovarian activity (Lightfoot et al., 1967; Hearnshaw et al., 1972; Adams, 1973; 1976b), anovulatory oestrus (Firth et al., 1977) and abnormal cycle lengths (Underwood and Shier, 1951; Obst and Seamark, 1970) is equivocal.

TEMPORARY INFERTILITY - When ewes grazed oestrogenic red clover over the mating period only, a depression in the incidence of multiple births and in conception rates was evident (Morley et al., 1964; Clark, 1965). However, fertility and fecundity were restored to normal levels within five weeks of removal of ewes from oestrogenic pasture (Morley et al., 1966). Chang (1963) noted a similar transient loss of fertility.

There is conflicting evidence as to whether the depression of multiple births is due to a depression in ovulation rate. Morley et al.,

(1964) and Holst and Braden, (1972) found no evidence of depressed ovulation rates. However, recent work has shown ovulation rates to be depressed in ewes mated on oestrogenic subterranean clover (Lightfoot and Wroth, 1974; Wroth and Lightfoot, 1976).

(b) Coumestans

In the early 1960's Coop and Clarke first reported a depression in the lambing percentage of ewes grazing lucerne at the time of mating. A similar response was reported in ewes mated on white clover (Sanger and Bell (1959). Coop and Clarke (1960) noted a dramatic decrease in twinning rate and a small, non-significant increase in the incidence of barrenness.

A number of subsequent trials have confirmed their findings, showing reductions in lambing percentages of up to 40 percent (Kelly et al., 1976a; McCutcheon, 1976; Scales and Moss, 1976; Scales et al., 1977). These trials are summarized in Table 1-2.

It was established that the decrease in lambing percentage, was due to a decrease in the number of multiple births and not to increased barrenness (Scales and Moss, 1976; McCutcheon, 1976; Scales et al., 1977). Furthermore, this depression in multiple birth rate was related to a reduction in ovulation rate and not to increased embryonic mortality (McCutcheon, 1976; Jagusch et al., 1977; Scales et al., 1977).

It is apparent that ewes of a higher inherent ovulation rate are most affected by coumestans (See Table 1-2).

There have been conflicting reports on the ability of the coumestans to inhibit or delay oestrus, and to induce anovulatory oestrus or follicular abnormalities. Kelly et al., (1976a) reported a high incidence of anovulatory oestrus and prolonged oestrus cycles, when they administered extremely high levels (c 1000 ppm) of coumestans. Moderately

TABLE 1-2 OESTROGENIC LUCERNE AND REPRODUCTIVE PERFORMANCE

	BREED	N	% DECREASE IN LAMBS BORN	% DECREASE IN MULTIPLE BIRTHS	% DECREASE IN OVULATION RATE	% INCREASE IN BARRENNESS
Coop & Clarke (1960)	Corriedales	370	6	6	-	0
	Border-Corr	242	15 *	11 *	-	2
	Romney	350	6	-	-	2
	Border-Corr	600	7 *	7 *	-	-
	Corriedales	610	5	7	-	0
Scales & Moss (1976)	Romneys	309	13	13	-	3
	Coopworths	539	23 *	32 *	-	1
McCutcheon (1976)	Border-Romn	80	8 *	23 *	23 *	2.5
Jagusch, Smith & Kelly (1977)	-	1000	18	-	More Single Ovul.	0
Coop (1977)	Border-Corr	900	-	20 *	-	2
Scales, Moss & Kelly (1977)	Romneys	309	16.4	13.7	-	2.7
	Coopworths	539	37.8 *	32.3 *	-	1.0
	Romneys	284	22.0	21.5	12	0.7
	Coopworths	146	27.9 *	23.6 *	24 *	5.1

* - Flocks of High Fecundity

high levels of coumestan, such as occur in field conditions (100-400ppm), apparently do not induce such abberations (Scales et al., 1977).

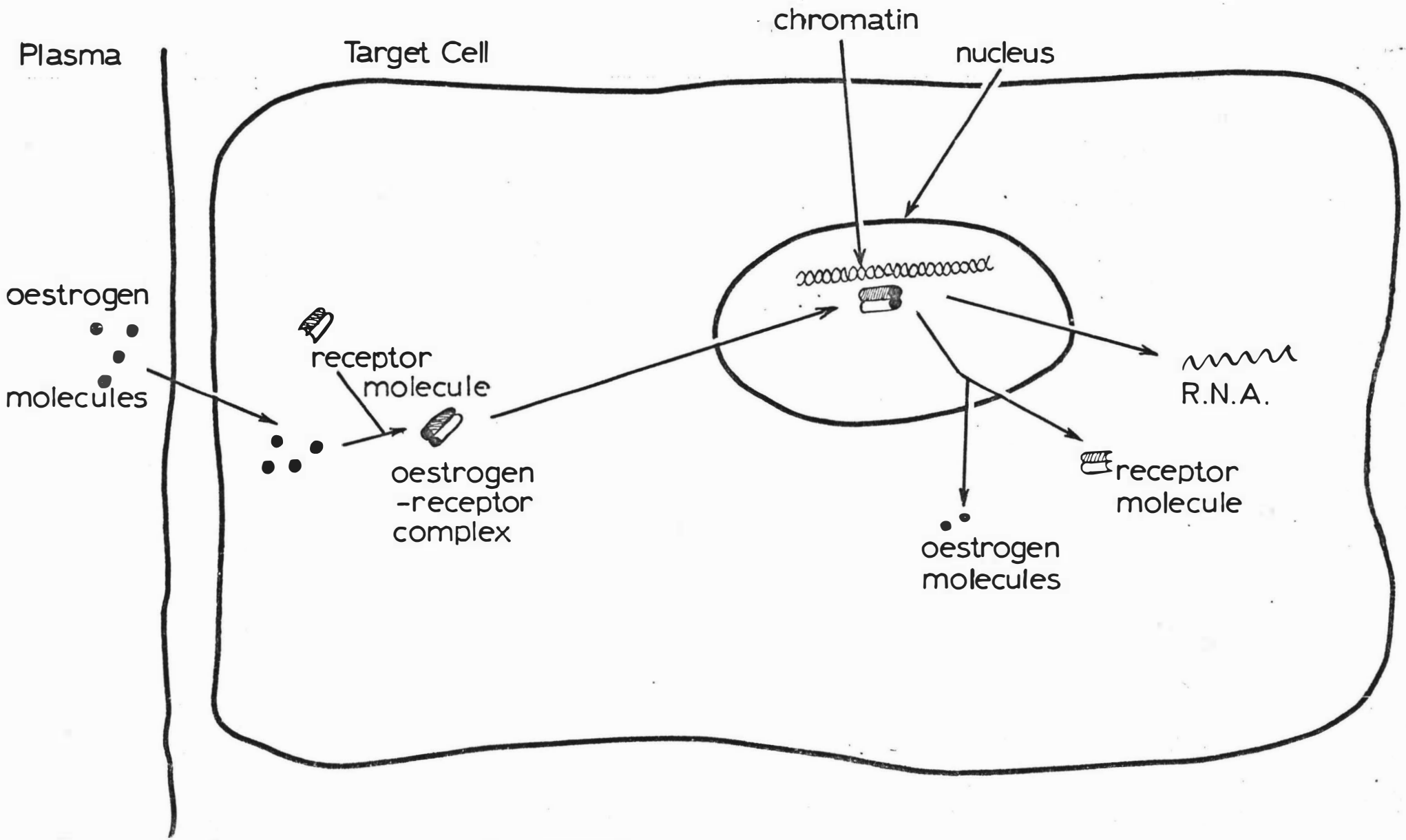
(B) THE MECHANISM OF ACTION OF OESTROGENS

The current model for oestrogen action in target tissues indicates that after entering a target cell, the hormone binds with high affinity to a cytoplasmic protein - the 'oestrogen receptor' - to form a complex. This complex undergoes rapid translocation to the nucleus, where it probably interacts with chromatin acceptor sites (Gorski and Gannon, 1976). Considerable evidence suggests that the presence of the receptor-oestrogen complex in the nucleus is a prerequisite for oestrogen-induced stimulation (Jenson and De Sombre, 1973). The nature of the oestrogen-induced biosynthetic events, depends not only on the quantity of receptor complexes within the nucleus, but also on the length of time the complexes stay within the nucleus (Anderson et al., 1975; Lan and Katzenellenbogen, 1976). Thus a single injection of oestriol, a weak oestrogen, can initiate only early biosynthetic events (0-6 hours) and is ineffective in stimulating later events (20-48 hours), presumably because of its high rate of dissociation from the receptor and its rapid clearance (Anderson et al., 1975). However, oestriol can be as effective as oestradiol in stimulating later responses if high circulating levels are maintained (Clark et al., 1977).

When oestriol is administered in acute doses, it acts as an oestrogen antagonist. It appears to compete with oestradiol for receptors (Clark et al., 1977). Oestrone apparently competes with oestradiol also (Wise and Payne, 1975).

A number of non-steroidal compounds act as anti-oestrogens, preventing the full expression of oestrogenic steroids on target tissues. Anti-oestrogens bind to oestrogen receptors (Capony and Rochefort, 1977) and induce translocation of receptors into the nucleus in the absence of

Fig. 1·3 POSTULATED MECHANISM OF OESTROGEN ACTION WITHIN TARGET CELLS



oestrogen, while inhibiting the translocation of receptor-oestrogen complexes (Ruh and Ruh, 1974). Receptor synthesis may also be blocked (Katzenellenbogen et al., 1977).

Paradoxically, in the absence of oestrogens, anti-oestrogens can stimulate some 'oestrogenic' responses (Fergusson and Katzenellenbogen, 1977). The effect of an anti-oestrogen is not identical in all target tissues, but differs between uterus, pituitary and hypothalamus (Luine and McEwen, 1977).

The postulated mechanism of action of oestrogens on target cells is outlined in Fig. 1-3.

(C) EXOGENOUS OESTROGENS AND REPRODUCTION IN THE EWE

(i) The Oestrous Cycle

Behavioural oestrus can be induced in anoestrous ewes by the administration of exogenous oestrogens (Moore and Robinson, 1957; Morley et al., 1963; Piper and Foote, 1968; Goding et al., 1970; Radford et al., 1970; Scaramuzzi et al., 1971a). The sensitivity of ewes to exogenous oestrogen is related to plasma progesterone levels (Moore and Robinson, 1957; Scaramuzzi et al., 1971b), and to breeding season (Reardon and Robinson, 1961; Fletcher and Lindsay, 1971a). The implication of oestrogens with behavioural oestrus has been confirmed in experiments involving the administration of antisera to endogenous oestrogens. Scaramuzzi (1975) and Fairclough et al., (1976) prevented the occurrence of behavioural oestrus by immunizing sheep against oestradiol. Cox and Wilson (1976) repeated this result with anti-oestradiol and anti-oestrone sera, but found that immunization against phyto-oestrogens did not prevent oestrus.

Scaramuzzi et al., (1971a) and Land et al., (1972) found a linear relationship between the dose of exogenous oestrogen and the duration of the induced oestrus. Land et al., (1972) suggested that a breed

difference in sensitivity to exogenous oestrogens exists; with highly fecund breeds being more sensitive in relation to this oestrus response. Scaramuzzi et al., (1972) found a refractoriness to oestrogen, with ewes showing a decline in response to repeated daily injections of oestradiol benzoate. Morley et al., (1963) administered stilboestrol daily to cycling ewes prior to and during mating, and reported decreased conception rates, and an increase in the incidence of silent heats.

Oestradiol injections given after day 8 of the oestrous cycle, result in premature luteolysis in intact ewes (Stormshak et al., 1969; Hawk and Bolt, 1970; Bolt and Hawk, 1971), but not in hysterectomized ewes (Stormshak et al., 1969). This luteolytic effect of oestrogen is possibly mediated through the stimulation of uterine prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Cox et al., (1974) and Barcikowski et al., (1974) reported increased PG $F_{2\alpha}$ secretion coinciding with endogenous oestrogen peaks on days 14-15 of the oestrous cycle. However, there was no coincidental $PGF_{2\alpha}$ rise with oestrogen peaks on days 4-5. Increases in $PGF_{2\alpha}$ secretion following the infusion of physiological doses of oestradiol into uterine plasma, have been noted (Barcikowski et al., 1974; Ford et al., 1975).

Obst and Seamark (1970) reported that plasma progesterone levels in sheep grazing oestrogenic clover, fell to 'oestrus' levels earlier than in sheep grazing pasture (days 14-15 compared with days 17-18). This supports the findings of Lightfoot and Wroth (1974) who reported that corpora lutea of clover-affected ewes weighed less than those of normal ewes. However, Hearnshaw et al., (1972) and Smith (1975) found clover infertile sheep to have normal progesterone secretion patterns.

Recently Newsome and Kitts (1977) measured receptor-bound oestrogen levels in ewes grazing lucerne and found that receptor-bound endogenous oestrogen levels were lower and more uniform than those of ewes grazing pasture.

(ii) Gonadotrophin Secretion

LUTEINIZING HORMONE - A relationship between steroid oestrogens and the pre-ovulatory LH surge was suggested as early as 1934, when Hohlweg found that oestrogen injections induced ovulation in rats. This 'Hohlweg effect' is also evident in the ewe (Hammond, 1945).

Moore et al., (1969) and Cox et al., (1971) demonstrated that the pre-ovulatory release of LH during oestrus in the ewe is primarily a response to high levels of circulating oestrogens the previous day. However, although oestradiol injections administered to cyclic ewes on days 3-4 of the oestrous cycle induced a pre-ovulatory type LH surge, injections of a similar dose level on days 10-12 did not (Bolt et al., 1971). Much higher dose levels are required at this time (Howland et al., 1971). These variations in response to exogenous oestrogens would appear to be related to plasma progesterone levels, however, there are conflicting reports on the ability of progesterone to block this oestrogen-induced LH surge (Yuthasastrakosol et al., 1974).
This pre-ovulatory-type LH surge can also be induced in anoestrous ewes (Goding et al., 1969; Radford et al., 1970; Beck and Reeves, 1973) and much smaller dose levels of exogenous oestrogens are required (Howland et al., 1971).

Exogenous oestrogens exert a somewhat more complex influence on ovariectomized ewes. Ovariectomy, and the subsequent decrease in plasma oestrogen levels, results in an elevation of basal LH levels in anoestrous ewes (Reeves et al., 1972; Diekman and Malvern, 1973). The administration of exogenous oestrogen to ovariectomised ewes induces a biphasic response in circulating LH levels - an initial depression followed by a large elevation, similar to pre-ovulatory levels (Radford et al., 1969; Goding et al., 1970; Scaramuzzi et al., 1971b; Howland and Palmer, 1973). Central nervous system blockade by sodium pentobarbitone anaesthesia, prevents only the second phase of the LH response (Radford and Wallace, 1974). This indicates a non-nervous site for the inhibitory action, and a

CNS site for the stimulatory action on LH release.

Not only is there variation in the sensitivity of ewes to exogenous oestrogen between the breeding season and the anoestrous period, but there is also variation in sensitivity between breeds. Finnish Landrace ewes are less sensitive than Scottish Blackface ewes (Land et al., 1976) to the negative-feedback effects of oestrogen, and this is suggested as a possible reason for variation in ovulation rates between breeds.

The ingestion of phyto-oestrogens disrupts these responses to exogenous oestrogens. Hearnshaw et al., (1972) found that ovariectomized ewes affected by 'clover disease' failed to produce any LH response to oestradiol injections. Synthetic Gonadotrophin releasing hormone (Gn-RH) does evoke the pre-ovulatory type LH response (Findlay et al., 1973). This suggests that prolonged exposure to isoflavones impairs hypothalamic receptors. Hearnshaw et al., (1977) postulated that 'temporary infertility' associated with isoflavones may also be related to the disruption of normal LH secretion patterns. They found that feeding oestrogenic clover to ovariectomized ewes, caused an initial depression then a rapid elevation of LH levels. Thus the response to oestrogenic clover paralleled the response obtained by administration of steroid oestrogens. There are no reports on the effects of coumestans on LH secretion patterns.

FOLLICLE STIMULATING HORMONE - Unilateral ovariectomy of cyclic ewes (Findlay and Cumming, 1977) or bilateral ovariectomy of cyclic or anoestrous ewes (Salamonsen et al., 1973), causes basal FSH levels to rise. This suggests that basal levels of FSH are regulated by oestrogen through a negative feedback system. As unilateral ovariectomy invokes a much greater response in FSH than in LH levels (Findlay and Cumming, 1977), it is suggested that FSH is more sensitive to negative feedback effects from the ovary than is LH - a situation which is also apparent during the

pre-ovulatory period in the intact ewe (Salamonsen et al., 1973; Baird and Scaramuzzi, 1976).

Infusion of oestradiol-17β into anoestrous ewes causes a dramatic increase in FSH release, coincident with LH release (Jonas et al., 1973; Salamonsen et al., 1973; Reeves et al., 1974; Pant, 1977) and in a similar pattern of secretion to that which follows administration of Gn-RH, except for an increased time lag (Jonas et al., 1973).

The addition of oestradiol to ovine pituitary cultures, even at extremely low concentrations, causes a dramatic decrease in FSH synthesis (Miller et al., 1977a; Steinberger and Chowdhury, 1977). However, the adminstration of oestradiol in vivo did not alter pituitary FSH levels (Steinberger and Chowdhury, 1977).

There are no reports on the effects of phyto-oestrogens on FSH secretion patterns.

GONADOTROPHIN RELEASING HORMONE - In 1964 Bognadove postulated that the variation in LH secretion throughout the oestrous cycle, was due to a change in pituitary responsiveness to Gn-RH. Gn-RH induces a greater change in serum LH (Reeves et al., 1971a) and FSH (Hooley et al., 1974) during an eight hour period on day 1, than at any other stage of the cycle. However, some workers have failed to find the variation in response (Foster and Crighton, 1974). A strong correlation between the oestradiol: progesterone ratio and the LH response to Gn-RH has been demonstrated (Thimonier et al., 1974; Pelletier, 1976). When progesterone levels are high, as in pregnancy, both FSH and LH responses to Gn-RH are reduced (Chamley et al., 1974a; 1974b). Similarly, daily progesterone injections suppress FSH and LH responses to Gn-RH (Hooley et al., 1974).

The administration of exogenous steroid oestrogens also affects pituitary responsiveness. Oestradiol benzoate administration to anoestrous ewes, increases the LH response to Gn-RH (Reeves et al., 1971b). More

precise studies have shown that oestrogens have a biphasic effect on pituitary responsiveness. When given a few hours (4-6) prior to Gn-RH, oestrogens depress the LH response, but an amplified effect of Gn-RH is observed if the time between oestrogen and Gn-RH treatments is extended (Pelletier and Signoret, 1970; Libertum et al., 1974). From changes in the FSH : LH ratio, it would appear that the inhibitory effect of oestrogen, influences FSH more than LH secretion (Libertum et al., 1974).

(iii) The Reproductive Tract

CERVICAL MUCUS SECRETION - Copious mucus secretion by the ovine cervix is generally attributed to oestrogen. Administration of oestradiol benzoate to ovariectomized ewes increases cervical mucus secretion (Moore and Robinson, 1957) and the response is linear (Allison, 1972). Rexroad and Barb, (1977) found that this increase in mucus production was not accompanied by any change in spinnbarkheit, chloride content or percentage dry matter.

The ingestion of phyto-oestrogens has also been shown to increase cervical mucus production for both isoflavones (Lindsay and Francis, 1968; Smith, 1971; Kelly et al., 1976b) and for coumestans (Kelly et al., 1976a). Adams (1976a) disputed that an increase in the amount of mucus produced did in fact occur, but that the visco-elasticity of the mucus was decreased by phyto-oestrogens. He showed that 'clover disease' ewes had lower spinnbarkheit (Adams, 1977). The sensitivity of ewes to exogenous steroid oestrogens is diminished by prolonged exposure to phyto-oestrogens (Adams, 1978).

OVA TRANSPORT AND FERTILIZATION - Exogenous oestrogens induce an acceleration in ova transport through the fallopian tubes (Chang, 1966; Restall, 1966). Holst and Braden (1972) found that phyto-oestrogens produced a similar effect.

The administration of oestradiol increases the rate and strength of uterine contractions markedly (Hawke, 1975) and also increases the number of contraction 'waves' moving towards the oviducts (Hawke, 1974). This improves sperm transport within the female tract, increasing the numbers of sperm found within the cervix, uterus and oviducts (Hawke and Cooper, 1975). This is in contradiction to the effects of the isoflavones on sperm transport. In sheep affected by clover disease, the number of sperm entering the cervix is considerably lower than normal (Turnbull et al., 1966). Apparently changes in the rheological properties of cervical mucus which depress sperm transport, outweigh any advantages from increased uterine motility.

Exogenous oestrogens apparently have no direct effects on fertilization or on embryo survival (Hawke and Cooper, 1976).

(iv) Oestrogen Receptors

Oestrogen receptor levels are replenished in the cytoplasm of target tissues by mechanisms which are partly dependant on protein synthesis. Oestrogens appear to be the most potent regulator of the dynamics of oestrogen-receptor turnover (Cidlowski and Muldoon, 1976; 1978). Receptor concentrations in ovine endometrium vary over the oestrous cycle. The pre-ovulatory surge of oestradiol promotes maximal receptor synthesis (Koligian and Stormshak, 1977b), hence the concentration of receptors is greatest at oestrus (Miller et al., 1977b). The concentration of receptors declines after oestrus, being lowest at the time of maximum progesterone synthesis (Miller et al., 1977b).

A single injection of exogenous oestradiol results in 60-75 percent of cytoplasmic receptors being translocated into the nucleus (Sutherland and Mester, 1976), the degree of translocation differing between target tissues (Morris, 1976). Continuous administration of exogenous oestrogen increases the concentration of cytoplasmic oestrogen receptors (Koligian and Stormshak, 1977a; Murphy et al., 1977), but progesterone suppresses

this oestrogen-induced increase (Hseuh et al., 1976).

Shemesh et al., (1972) demonstrated that phyto-oestrogens also bind to oestrogen receptors. Both coumestrol and genistein compete with oestradiol for receptors. Chamley et al., (1974c) found a greater concentration of oestradiol-receptor complexes in 'clover disease' ewes. This suggests that phyto-oestrogens are also able to induce the synthesis of receptors.

(D) FOLLICLE MATURATION IN THE EWE

(i) Patterns of Follicle Growth and Atresia

Follicular growth is described as a continuum. It proceeds at all times, at all ages, uninterrupted by pregnancy, anoestrous or other periods of non-ovulation (Peters et al., 1975; Matton et al., 1977).

In the ewe, the numbers of follicular growth 'waves' during the oestrous cycle have been variously reported as one (Hutchinson and Robertson, 1966), two (Brand and De Jong, 1973), and three or four (Smeaton and Robertson, 1971). Recently Turnbull et al., (1977a) disputed the existence of any 'waves' of follicular growth, and suggested that follicles are growing and regressing asynchronously at any given time of the oestrous cycle.

The initiation of growth of primordial follicles is a continuous process in ewes (Peters et al., 1975), and the number of 'initiated' follicles which finally ovulate is determined by the rate of atresia during their growth phase (Hay and Moor, 1975b; Turnbull et al., 1977a).

Turnbull et al., (1977a) estimated follicle growth rates by the mitotic index of granulosa and thecal cells. They found that irrespective of the stage of the cycle, the growth rate was slow in follicles of up to 0.4mm diameter, after which it accelerated until it reached a maximum in follicles of about 0.7mm diameter. This fast rate was maintained until

the follicles reached a diameter of about 2mm and then declined by about 50 percent. In sheep, those follicles destined to ovulate would have a diameter greater than 0.5mm on day 6-7 of the cycle, although not all follicles of this diameter would ovulate. In Merinos with an ovulation rate of 1 to 2, about 3-4 follicles enter the rapid growth phase each day (Turnbull et al., 1977a). In single ovulator breeds, follicles of a diameter greater than 1mm have a slower growth rate than do follicles of a similar size range in multiple ovulator breeds (Turnbull et al., 1977b). Thus for the same total number of follicles present, the multiple ovulator will have a greater number capable of ovulation, when compared with a single ovulator (Dufour and Matton, 1977; Dufour, 1976). The number of follicles which actually do ovulate, is apparently determined within the 3 days prior to oestrus (Land, 1973; Findlay and Cumming, 1977; Bherer et al., 1977).

(ii) Hormonal Control of Follicle Growth and Atresia

GONADOTROPHINS - The initiation of growth of primordial follicles appears to be independant of gonadotrophin levels (Lunenfield et al., 1975; Peters et al., 1975). Two intra-ovarian factors have been demonstrated to influence the initiation of follicular growth; (a) the size of the pool of non-growing follicles - a reduction in pool size causes fewer follicles to start growing (Krarup et al., 1969) (b) the degeneration of large follicles - large follicles apparently release a substance that suppresses growth initiation (Peters et al., 1973).

There is evidence that the administration of gonadotrophins (PMSG) to ewes, will increase the number of 'initiated' follicles entering the rapid growth phase, prevent atresia in large follicles (Hay and Moor, 1975b; Turnbull et al., 1977a), and increase ovulation rates (Cumming and McDonald, 1967). Conversely, the reduction of FSH and LH levels by the administration of antisera, acutely prevents the development of

small follicles to Graafian follicles (Welchen and Dullaart, 1976), and even the temporary absence of LH results in follicle atresia (Schwartz, 1974).

Although a direct relationship has not been established between FSH levels and ovulation rate (Findlay and Cumming, 1976a), there is considerable indirect evidence for such a relationship. Unilateral ovariectomy results in compensatory follicular growth and in ovulation rates, in the remaining ovary (Sundaram and Stob, 1967; Mallampati and Casida, 1970; Dufour et al., 1971a; Land, 1973; Findlay and Cumming, 1977). This compensatory growth is apparent only if hemicastration is carried out prior to 3 days before the onset of oestrus. There is a transient increase in FSH, and to a lesser degree in LH, levels after hemicastration which coincides with falling oestradiol levels (Ramirez and Sawyer, 1974; Findlay and Cumming, 1977). Oestrogen replacement therapy suppresses both the rise in FSH levels and compensation in follicular growth and ovulation following hemicastration (Ramirez and Sawyer, 1974). 'Clover disease' ewes also fail to show expected ovarian compensatory hypertrophy (Adams, 1976b).

The increase in ovulation rates in ewes fed lupin grain (Lightfoot et al., 1976) is also accompanied by increased FSH levels (Brien et al., 1976).

Trounson et al., (1974) reported that lambs selected on multiple birth characteristics of their dam, had a greater number of primordial follicles than did control lambs. They suggested that this may be related to higher endogenous gonadotrophin levels. However, Land et al., (1973) and Echternkamp and Laster, (1976) were unable to find any relationship between ovulation rates and endogenous LH levels. Bindon et al., (1971) reported a greater ovarian response to exogenous gonadotrophins by high fecundity ewes than by low fecundity ewes. Land et al., (1972, 1976) and Land (1976) have shown that high fecundity breeds are less sensitive to the effects of exogenous oestrogens on gonadotrophin release.

STEROID HORMONES - All follicles, including those in various stages of atresia, secrete a range of steroids including oestrogens, androgens and progestins (Seamark et al., 1974). The amounts and precise pattern of steroid secretion of an individual follicle varies according to its developmental stage. The smaller follicles produce predominantly androgens, whereas the larger follicles produce both oestrogens and androgens (Seamark, 1978). Atretic and non-atretic small follicles are equally active steroidogenically.

Ovarian oestrogen secretion rises from baseline levels on day 13 of the cycle to a peak on day 15, and then declines rapidly at oestrus (Moor et al., 1971; Moor 1974; Edwards, 1974). These high levels of oestrogens are secreted almost entirely by the largest one or two non-atretic follicles (Bjersing et al., 1972; Moor, 1973; Baird et al., 1975; Hay and Moor, 1975b). The enzyme Δ^5 $^3\beta$ hydroxysteroid dehydrogenase plays a key role in oestrogen synthesis (Moor et al., 1971) and although it's activity appears to be confined to the thecal cells (Hay and Moor, 1975a), interaction between thecal and granulosa cells is apparently required for oestrogen production (Moor, 1977). It is not known what initiates this surge in oestrogen production, but exogenous gonadotrophin (PMSG) induces increased activity of this enzyme (Moor et al., 1971). The pre-ovulatory rise in LH terminates this surge in oestrogen production (Moor, 1974). The Graafian follicle is extremely sensitive to minor changes in basal LH concentrations (Baird et al., 1976) and variation in the secretion of oestradiol-17β probably reflects the response to small pulses in LH (Wheeler et al., 1977a).

As a consequence of the pre-ovulatory changes in circulating gonadotrophins, oestrogen production by the large non-atretic follicles is reduced and replaced by an increase in the release of androgens and progestins. Twelve to 18 hours prior to ovulation, the large follicles

produce little or no oestrogen, but large amounts of progesterone and 20α pregn-4-en-3-one (Seamark, 1978). It has been suggested that progesterone may have an effect on the ovary independent of its influence via the hypothalamo-pituitary axis (Rexroad and Casida, 1977). Dufour et al., (1971b) reported that follicle growth was more rapid in ovaries that contain a corpus luteum. Injection of progesterone into one ovary of anoestrous ewes, with concurrent systemic administration of PMSG, results in more rapid follicular growth, although oestrogen secretion is unchanged, (Rexroad and Casida, 1977) and in a greater number of follicles and corpora lutea in the injected, than in the control ovary (Harned and Casida, 1971). Progesterone may act to alter time-dependent changes in follicle size, thus enabling some follicles to grow while others undergo atresia.

HORMONE RECEPTORS - It has been proposed that the selection of those follicles destined to ovulate, is a consequence of both sequential changes in follicular hormone receptor levels and in circulating pituitary gonadotrophin levels (Carson et al., 1978).

As follicular growth proceeds, FSH receptor levels decrease and LH receptor levels increase within granulosa cells (Channing, 1970; Carson et al., 1978), but remain unchanged within thecal cells (Weiss and Armstrong, 1977; Carson et al., 1978). FSH has been shown to increase numbers of FSH receptors; oestradiol to increase oestradiol receptors; and FSH and oestradiol in concert to increase LH receptors, in granulosa cells (Richards and Midgley, 1976). Termination of follicular growth by LH-induced luteinization is associated with the loss of receptors for FSH and oestradiol, and the subsequent increase in LH receptors (Richards and Midgley, 1976).

(E) OVULATION IN THE EWE

(i) Factors Influencing Ovulation Rates

Heape (1899) first reported that heavy ewes produced more lambs than light ewes. This is related to a higher production of ova (Wallace, 1961; Allen and Lamming, 1961; Killeen, 1967; Allison, 1968). This correlation between bodyweight and ovulation rate has been described by Edey (1968) who found a linear relationship within a particular weight range. Ducker and Boyd (1977) demonstrated that it is the body condition element of ewe liveweight, not body size, that influences ovulation rates. However, Cumming (1977) showed that liveweight alone was as accurate a predictor of ovulation rate as both liveweight and body condition.

Several workers have reported evidence of a dynamic effect of flushing, independent of bodyweight, on ovulation rate (Coop, 1966a; 1966b; McInnes and Smith, 1966; Killeen 1967) although others have found no effect (Hulet et al., 1968; Fletcher, 1971; Cumming, 1977).

There is considerable variation in fecundity between breeds and between strains within a breed. Ewes of higher fecundity (Trounson et al., 1974) and their daughters (McDonald and Chang, 1966) ovulate a greater number of follicles, and a higher proportion of the Graafian follicles that develop (Bradford et al., 1971; Turnbull et al., 1977b). Fecundity and ovulation rates are not related to LH secretion throughout the cycle (Echternkamp and Laster, 1976) nor to LH peaks (Land et al., 1973). There is some indirect evidence that FSH levels around days 12-14 are important in determining ovulation rates at the subsequent oestrus, (Cumming and McDonald, 1967; Brien et al., 1976; Findlay and Cumming 1976b; 1977) but there are no reports of a direct relationship between FSH levels and fecundity. Liveweight or flushing apparently does not influence FSH levels (Findlay and Cumming, 1976a).

Wheeler et al., (1977b) showed that mean secretion rates of oestradiol-17β are significantly higher in ewes with high ovulation rates, and Land et al., (1976) found such ewes less sensitive to exogenous oestradiol-induced depression of ovulation rates. Ewes of high fecundity also show a greater response to PMSG-induced superovulation (Eastwood and McDonald, 1975).

It has been suggested that the length of the interval from the onset of oestrus to the time of ovulation may influence ovulation rate. Land (1970) and Hanrahan and Quirke (1975) showed a positive relationship between the duration of oestrus and ovulation rate. Cumming et al., (1973) demonstrated a high degree of constancy in the interval from the pre-ovulatory LH peak to ovulation. Lindsay et al., (1975) found that the presence of rams during oestrus induced ovulation earlier by advancing the time of the LH surge. There appears to be a considerable variation in the interval from the onset of oestrus to the LH surge (Lamming pers com., 1978).

(ii) Postulated Mechanisms of Ovulation

The precise process, or processes, of ovulation remain obscure although considerable effort has been put into identifying them. Two major types of ovulatory mechanisms have been suggested; those involving 'enzymatic breakdown' of follicular tissue and those involving the 'bursting' of the follicle due to pressure changes.

There is substantial evidence suggesting that the collagenous connective tissue of the wall of the Graafian follicle is degraded near the time of ovulation (Espey, 1974). Collagenolytic enzymes have been detected within ovarian follicles; both neutral and acid proteases, capable of degrading collagen, are produced in mature Graafian follicles (Espey and Coons, 1976).

Although the growth and maturation of follicles occurs without a measurable increase in hydrostatic pressure (Rodbard, 1968) a follicular contractile process has been demonstrated. Electron microscopy shows many cells in the theca externa of mature sheep follicles, with cytoplasmic filaments and dense bodies characteristic of smooth muscle cells (O'Shea, 1971). Strips of follicular tissue exhibit classical contractile activity, (O'Shea and Phillips, 1974) and autonomic innervation, both adrenergic and cholinergic, is associated with follicles (Bahr et al., 1974; Owman et al., 1975; Walles et al., 1976; 1977). Perfused ovarian tissue has been shown to be electrically active (Tojo et al., 1975).

It has been suggested that prostaglandin $F_{2\alpha}$ is involved in the induction of ovulation (Tsafriri et al., 1972). Concentrations of prostaglandins increase within the developing follicle late in the oestrous cycle, most dramatically in those follicles which eventually ovulate (Yang et al., 1974). This rise in prostaglandin levels is apparently induced by the surge in endogenous oestradiol which occurs at days 14-15 of the cycle, but not by earlier oestradiol peaks (Barcikowski et al., 1974; Cox et al.1974).Infusion of physiological doses of oestradiol into the uterus on days 14-15 will induce comparable prostaglandin peaks (Barcikowski et al., 1974).

Systemic or intrafollicular injection of prostaglandin $F_{2\alpha}$ induces ovulation (Phi et al., 1977) and similarly perfusion of follicles 'in vitro' enhances ovulation (Hamada et al., 1977). This induction of ovulation is blocked by known prostaglandin $F_{2\alpha}$ antagonists such as prostaglandin E_2 and indomethacin (Carlson et al., 1974).

Yang et al., (1974) have suggested that prostaglandin $F_{2\alpha}$ may be involved in the synthesis, release or activation of collagenolytic enzymes and Tojo et al., (1975) has shown that prostaglandin $F_{2\alpha}$

accelerates the electrical activity of perfused ovarian tissue.

Intrafollicular injection of LH (and in some experiments, of FSH) will induce ovulation (Jones and Nalbandov, 1972). Most evidence indicates that LH is the sole agent required to trigger ovulation, while both FSH and LH are required for the development, maturation and luteinization of follicular tissue (Rondell, 1974). It has been well established that LH induces increased synthesis of cyclic AMP and subsequently, increased protein synthesis (Robison et al., 1971). Prostaglandins are capable of increasing cAMP formation (Marsh, 1971) and conversely anti-prostaglandin agents block the stimulatory action of LH on cAMP synthesis (Kuehl et al., 1970). Protein synthesis appears to be an essential part of the metabolic processes supporting ovulation, as inhibitors of protein synthesis inhibit LH-induced ovulation (Lipner and Greep, 1971).

(iii) Blockade of Ovulation

It has been well established that indomethacin blocks ovulation in ewes (Carlson et al., 1974; Armstrong et al., 1974; Lewis and Warren, 1975). Indomethacin blocks the LH-induced increase in prostaglandin $F_{2\alpha}$ levels in ovarian follicles (Armstrong et al., 1974) and inhibits the oestradiol-induced LH release in anoestrous ewes (Carlson et al., 1974). Similarly the administration of inhibitory analogues of LH-RH will also effectively block ovulation (Phelps et al., 1977).

This complete blockade of ovulation is not apparent in ewes ingesting coumestans. Oestrogenic lucerne reduces the number of ovulations, but seldom prevents ovulation completely.

(F) THE PURPOSE AND SCOPE OF THE INVESTIGATION

It has been clearly established that the ingestion of oestrogenic lucerne by ewes, depresses ovulation rates. Because of the important role of the gonadotrophins in the maturation and ultimate ovulation of ovarian follicles, it is possible that the coumestans could alter the pattern of gonadotrophic secretion. FSH is involved in the development of Graafian follicles, and LH facilitates the final stages of maturation and the induction of ovulation. The coumestan-induced depression of ovulation rate may arise from a decreased pituitary output of these gonadotrophins, or from changes in the pattern of their release, particularly near the time of oestrus.

The present trials investigated the effects of oestrogenic lucerne, fed over the oestrous cycle, on LH secretion patterns, and on the timing of the pre-ovulatory LH-surge in relation to oestrus and ovulation (Experiment I). Ovarian activity was recorded as a measure of gonadotrophic function. In Experiment II, the effects of oestrogenic lucerne on ovarian activity, were studied when lucerne was fed early or late in the oestrous cycle, with the view of determining the critical time involved in the depression of ovulation rates.

C H A P T E R I I

MATERIALS AND METHODS

CHAPTER II

MATERIALS AND METHODS

(A) EXPERIMENTAL PLAN

Two separate trials were carried out to investigate the effects of oestrogenic lucerne on cycling ewes. In the first experiment plasma gonadotrophin concentrations were measured over a complete oestrous cycle, and in the second experiment the effects of coumestan intake either early or late in the oestrous cycle were recorded. The experimental procedures and calender of events are set out in FIGS. 2-1 and 2-2.

EXPERIMENT I - 22 mixed aged Romney ewes and 20 mixed aged Border Leicester x Romney ewes were randomly assigned to two groups with equal numbers of each breed. At the detection of oestrus following synchronization, each group grazed either oestrogenic lucerne or non-oestrogenic ryegrass/clover pasture.

Blood samples were collected from all ewes by jugular venepuncture. Samples were taken daily for 17 days, from day 7 of experimental oestrous cycle. On predicted day 16 of the cycle, blood samples were taken at 4-hourly intervals until the detection of oestrus; then at 2-hourly intervals for 24 hours and at 4-hourly intervals for the subsequent 12 hours.

All ewes underwent laparotomy 3 days post-oestrus at which time corpora lutea and surface follicles were recorded.

Over the period of intensive blood sampling, the animals were kept in small holding paddocks and yards adjacent to laboratory facilities (Massey University Fertility Centre). Ewes in the lucerne group were

Fig.2·1 EXPERIMENTAL PLAN Expt. 1

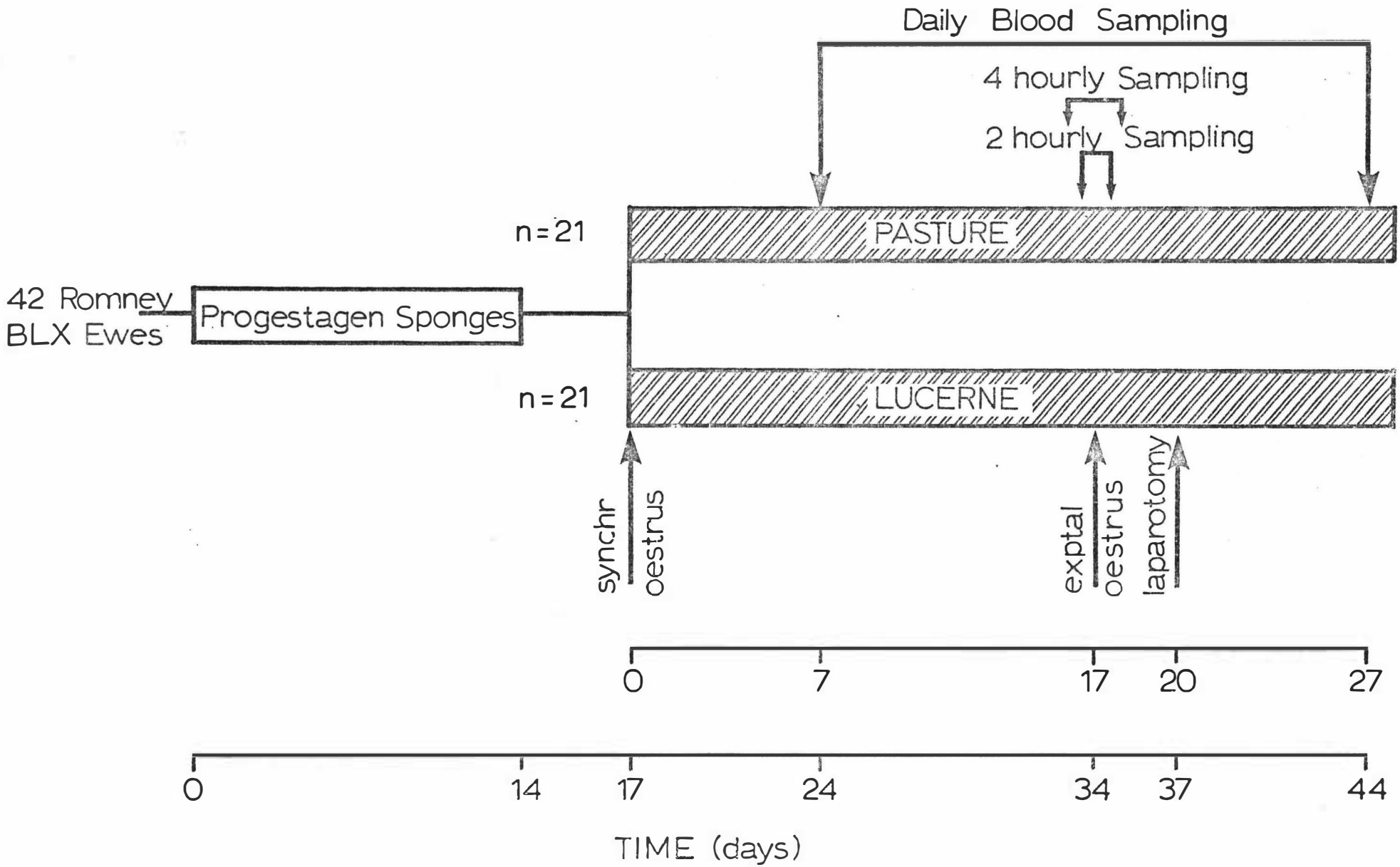
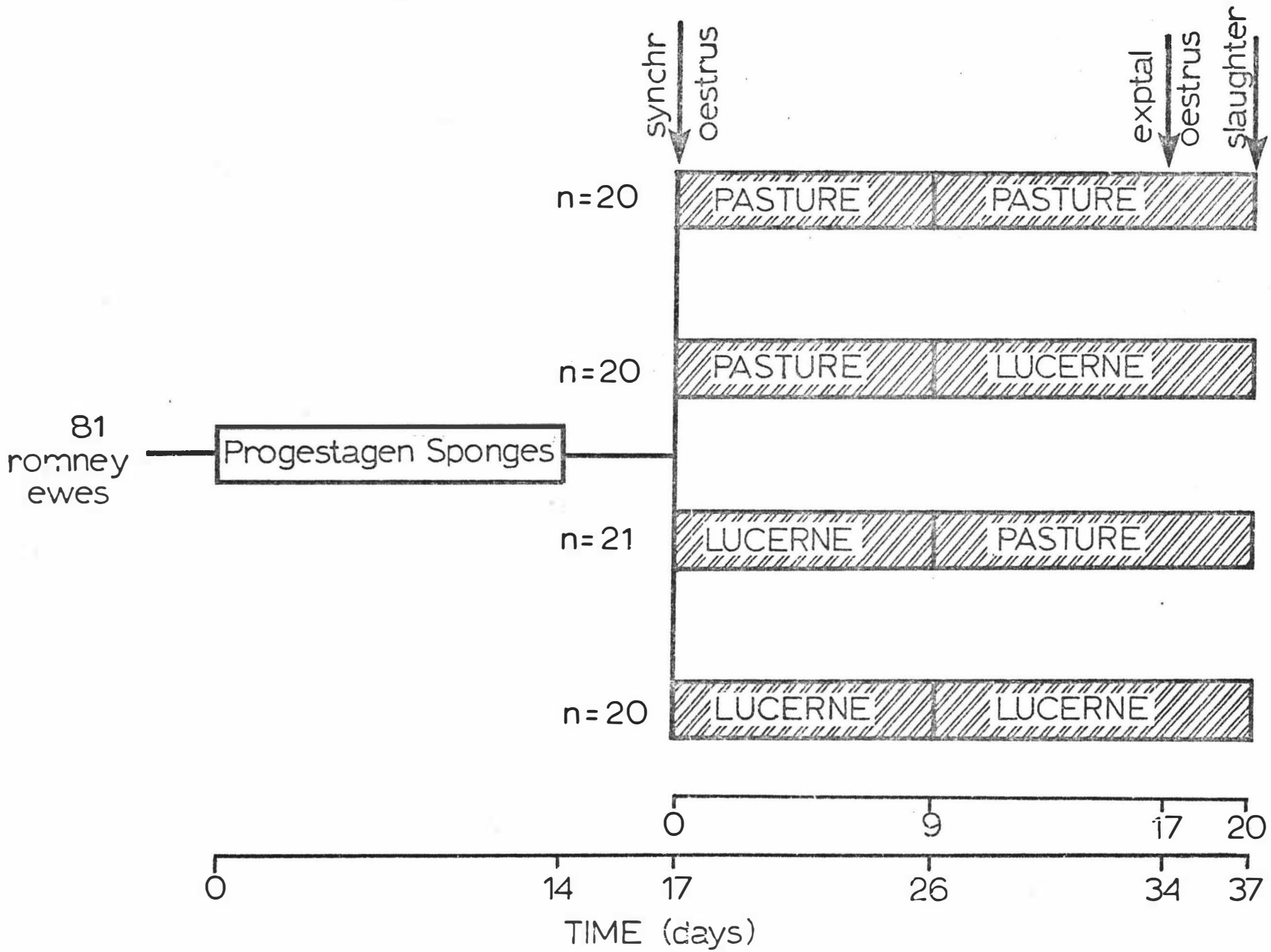


Fig.22 EXPERIMENTAL PLAN Expt. 2



fed fresh lucerne cut daily from the crop.

EXPERIMENT II - 81 mixed aged Romney ewes were randomly assigned to four groups. At the detection of oestrus following synchronization, the groups were assigned to one of four grazing regimes; pasture throughout the oestrous cycle, lucerne throughout the cycle, or these treatments were interchanged at midcycle. In the latter two groups treatments were changed on day 9 of the experimental oestrous cycle.

All ewes were slaughtered 3 days post-oestrus at the local freezing works (Borthwicks - CWS, Longburn). Reproductive tracts were recovered within 15 minutes of slaughter and individually identified.

(B) LUCERNE AND CONTROL PASTURES

The lucerne crop involved in these experiments consisted of two hectares of Wairau lucerne on a Rangitikei Loamy sand. The paddock was sub-divided into four plots of approximately 0.5ha. Experimental grazing commenced eight weeks after the lucerne had been previously harvested, at which time plant heights ranged from 60 to 90cms.

Approximately 25 percent of plants were in flower and there was moderately heavy fungal infection of plant tissue (more than 5 lesions per leaf).

A three hectare ryegrass/white clover pasture on a Manawatu Fine Sandy Loam adjacent to the lucerne stand was grazed by control animals.

Leaf samples of lucerne, and of white clover, were taken weekly and assayed for coumestan content. This involved random selection of material from 40 plants from each plot.

(C) ANIMALS

The animals utilized were 103 mixed-age Romney ewes and 20 mixed-aged F_1 Border Leicester X Romney ewes, drawn from several flocks

maintained on Massey University farms. All ewes were identified by numbered brass eartags and by midside brands, which were applied at the commencement of the trial.

All ewes were weighed prior to, and at the completion of oestrus synchronization treatment, as well as at the completion of the trial. Ewes in Experiment I were also weighed two weeks prior to synchronization treatment. Attempts were made to carry out all liveweight recordings at the same hour of the day, in order to minimise variations in gutfill.

(D) OESTRUS SYNCHRONIZATION

In order to synchronize oestrous cycles, all ewes were subjected to 14-day treatments with polyurethane intravaginal sponges containing 40mg of methyl-acetoxy-progesterone (M.A.P.). Vasectomized rams fitted with Sire Sine harnesses were used to detect oestrus following pessary removal.

(E) HORMONE ANALYSES

COUMESTANS - Lucerne and clover samples were subjected to ethanol extraction (Kelly, 1976) and extracts were forwarded to Dr. R.W. Kelly, Invermay Agricultural Research Centre, for analysis of coumestan content.

PLASMA GONADOTROPHINS - Blood samples were assayed for ovine luteinizing hormone by radioimmunoassay, following the double-antibody technique of Niswender et al., (1969), modified according to Barrell (1976). This assay utilized the following materials: rabbit anti-ovine LH serum (pool 15 courtesy Dr G.D. Niswender), NIH-LH-S18 as assay standards, and highly purified ovine LH (LER-1374A) for radioiodination. Assay standards and samples were assayed in triplicate, with hypophysectomized

sheep plasma added to standard curve tubes. Mean assay sensitivity was 0.2ng/ml, and the intra-and inter-assay coefficients of variation (CV) were 10.3 and 23% respectively.

At the time of planning of these experiments, a supply of FSH-antisera became available. On the assumption that this assay would be in operation, FSH analyses were to be included in experimental procedures. However, with the immunochemicals available, binding of iodinated FSH was inadequate, and consequently this assay was omitted from plasma analyses.

(F) CERVICAL MUCUS ANALYSIS

Cervical mucus samples were taken from all ewes in Experiment I at blood collection times over the oestrus period. The rams used for detection of oestrus were fitted with canvas 'aprons' to prevent penile intromission and subsequent contamination of cervical mucus. Mucus samples were collected, with the aid of a speculum, using absorbent cotton swabs.

Cervical mucus samples were assessed for chloride ion content by the method of Turnbull et al., (1967). Silver chromate was precipitated onto strips of Whatman No. 1 chromatography paper, by impregnating the paper, first with 0.275 N silver nitrate and then with 0.175 potassium chromate. Chloride ions from the mucus samples produce white to buff-coloured spots, which are compared to sodium chloride standards. Oestrus is associated with a rise in chloride concentrations, and ovulation with a sudden fall in chloride levels.

(G) UTERINE AND OVARIAN WEIGHTS

After collection at slaughter, uteri were separated from the tract by transection immediately anterior to the cervix, dried with paper towels and weighed.

Ovaries from each ewe were weighed separately. Where part of the uterus, or part of an ovary, was lost at slaughter, that animal was omitted from the analysis.

(H) FOLLICLE POPULATIONS

After collection at slaughter, the ovaries were preserved in Bouins fluid until sectioning and assessment of follicle populations. To assess the total ovarian follicular population, all ovaries were sectioned into 1mm slices. Follicle diameter was calculated as the mean of two measurements taken at right angles to each other. Counts were made of all antral follicles with a dimaeter of 2mm or greater.

(I) REPRODUCTIVE TRACT HISTOLOGY

At the time of recovery of the reproductive tract, 1cm sections of vagina, cervix, uterus and fallopian tube were taken and preserved in Bouins Fluid for later histological examination.

Sections of 6μ thickness, were taken of all tract specimens and stained with Haematoxylin and Eosin. The mean height of epithelial cells was calculated for all sections, by taking 25 separate measurements from each section.

(J) ANALYSIS OF DATA

Analyses of discrete data - numbers of ovulations and follicle populations - utilized Chi^2 statistic.

Data involving:

Ewe liveweights

LH concentration in peripheral plasma

Ovarian weights

Epithelial cell heights

were analysed by analysis of variance (Snedecor and Cochrane, 1967).

Data from 8 ewes were excluded from the analyses of LH concentrations and ovulation rates owing to either failure of oestrus synchronization (4 ewes) or failure to detect the pre-ovulatory LH peak (4 ewes). Data from 9 ewes were excluded from Expt. II due to failure of heat detection for a variety of reasons; Sponges lost (4 ewes), blind tracts (2 ewes), silent heat (2 ewes) and pregnancy (1 ewe). Data from a further 12 ewes were excluded from reproductive tract analyses, due to cystic hyperplasia (1 ewe), anovulatory oestrus (2 ewes), or incomplete recovery of the tract at slaughter (9 ewes).

CHAPTER III

RESULTS

C H A P T E R I I I

RESULTS

(A) LUCERNE OESTROGENIC POTENCY

(i) Lucerne Coumestan Content

Mean levels of the coumestans, coumestrol and 4-methoxycoumestrol, for each of the four plots and for clover samples, are shown in Table 3-1. Values are given as parts per million on a dry matter basis.

TABLE 3-1: LUCERNE COUMESTAN CONTENT (ppm)

DATE	PLOT A	PLOT B	PLOT C	PLOT D	CLOVER
6: 4: 77					
Coumestrol	98	151	66	100	< 10
4-Methoxycoumestrol	60	106	33	77	< 10
13: 4: 77					
Coumestrol	69	122	81	110	< 5
4-Methoxycoumestrol	34	70	41	63	< 5
20: 4: 77					
Coumestrol	88	94	145	172	< 5
4-Methoxycoumestrol	38	59	107	145	< 5

(ii) Daily Coumestan Intake

Sheep are capable of consuming each day, a total weight of forage equal to 3 percent of their bodyweight. Considering that lucerne, during its flowering stages, is approximately 24 percent dry matter, then a conservative estimate of the daily intake of lucerne by ewes in

this trial, would be 1kg of dry matter. The mean daily intake of coumestans, for ewes in both experiments, based on a daily intake of 1kg of dry matter, are shown in Table 3-2.

TABLE 3-2. THE DAILY COUMESTAN INTAKE (mg/day) OF EWES INGESTING 1KG (DM) OF LUCERNE

EXPERIMENTAL GROUP	STAGE OF OESTROUS CYCLE (DAYS)		
	0-9	10-17	18-25
EXPERIMENT I			
Pasture	2	2	1
Lucerne	117	252	173
EXPERIMENT II			
Pasture	1	1	-
Lucerne	192	217	-
Past-Luc	1	217	-
Luc-Past	192	1	-

The coumestan intake of ewes grazing ryegrass/clover pasture, is based on the assumption that white clover accounts for 10 percent of daily dry matter intake.

(B) EWE LIVEWEIGHTS AND OESTRUS

(i) Ewe Liveweights

Mean liveweights of ewes in both experiments are listed in Table 3-3. There were no significant differences between treatment groups at any of the weight recordings. Similarly there were no significant differences in the rate of weight gain between weighings.

TABLE 3-3: MEAN EWE LIVEWEIGHTS (kg)

EXPERIMENT I

	PASTURE	LUCERNE
NUMBER OF EWES	21	21
TIME OF WEIGHING:		
Pre-Experimental	57.00 ± 1.0	56.70 ± 1.53
Sponge Insertion	61.50 ± 0.95	60.40 ± 1.31
Sponge Withdrawal	58.50 ± 1.01	57.90 ± 1.35
Post-Experimental	61.60 ± 1.19	60.00 ± 1.31

EXPERIMENT II

	PASTURE	LUCERNE	PAST-LUCERNE	LUCERNE-PAST
NUMBER OF EWES	20	21	20	20
TIME OF WEIGHING :				
Sponge Insertion	57.70 ± 1.61	56.00 ± 1.32	57.00 ± 1.58	58.60 ± 0.99
Sponge Withdrawal	61.60 ± 1.86	60.70 ± 1.44	61.50 ± 1.53	63.20 ± 0.99
Post Experimental	63.80 ± 1.72	62.00 ± 1.30	63.20 ± 1.51	63.90 ± 1.15

(ii) Oestrus Synchronization

The distribution of the onset of oestrus for both the synchronized and the experimental oestrus are shown in FIGS 3-1 and 3-2.

In Experiment I, 93 percent of the ewes were detected in oestrus over a four day period following sponge withdrawal. Eighty two percent of the ewes were detected in heat over a corresponding four day period in the following oestrous cycle.

In Experiment II, 83.3 percent of the ewes were detected over a four day period for the synchronized oestrus and 76.5 percent in the following oestrus. Inspection of the reproductive tracts later revealed that a number of ewes were physiologically unable to exhibit oestrus. Two ewes had blind reproductive tracts with only vaginal tissue present, and one ewe was found to be pregnant. A further four ewes had lost sponges during synchronization. Only two ewes exhibited 'silent' heats, one in the pasture group and one in the lucerne-pasture group.

There were no significant differences in oestrus synchrony at the second oestrous cycle between ewes grazing lucerne and ewes grazing pasture. Similarly there were no differences in mean cycle length.

(iii) Oestrus-Ovulation Interval

The interval between the onset of oestrus and the pre-ovulatory LH peak, was extremely variable between animals, in both treatment groups. Some animals were recorded as only entering oestrus, after the LH peak had occurred. The interval length for individual sheep is shown in FIG 3-3. There were no significant differences between ewes grazing lucerne and ewes grazing pasture in the mean interval duration.

There were no apparent patterns shown in the chloride content of cervical mucus samples collected. Chloride concentrations appeared to rise and fall randomly. Consequently, the time of ovulation could not

Fig. 3·1 DISTRIBUTION OF THE ONSET OF OESTRUS – Expt. I

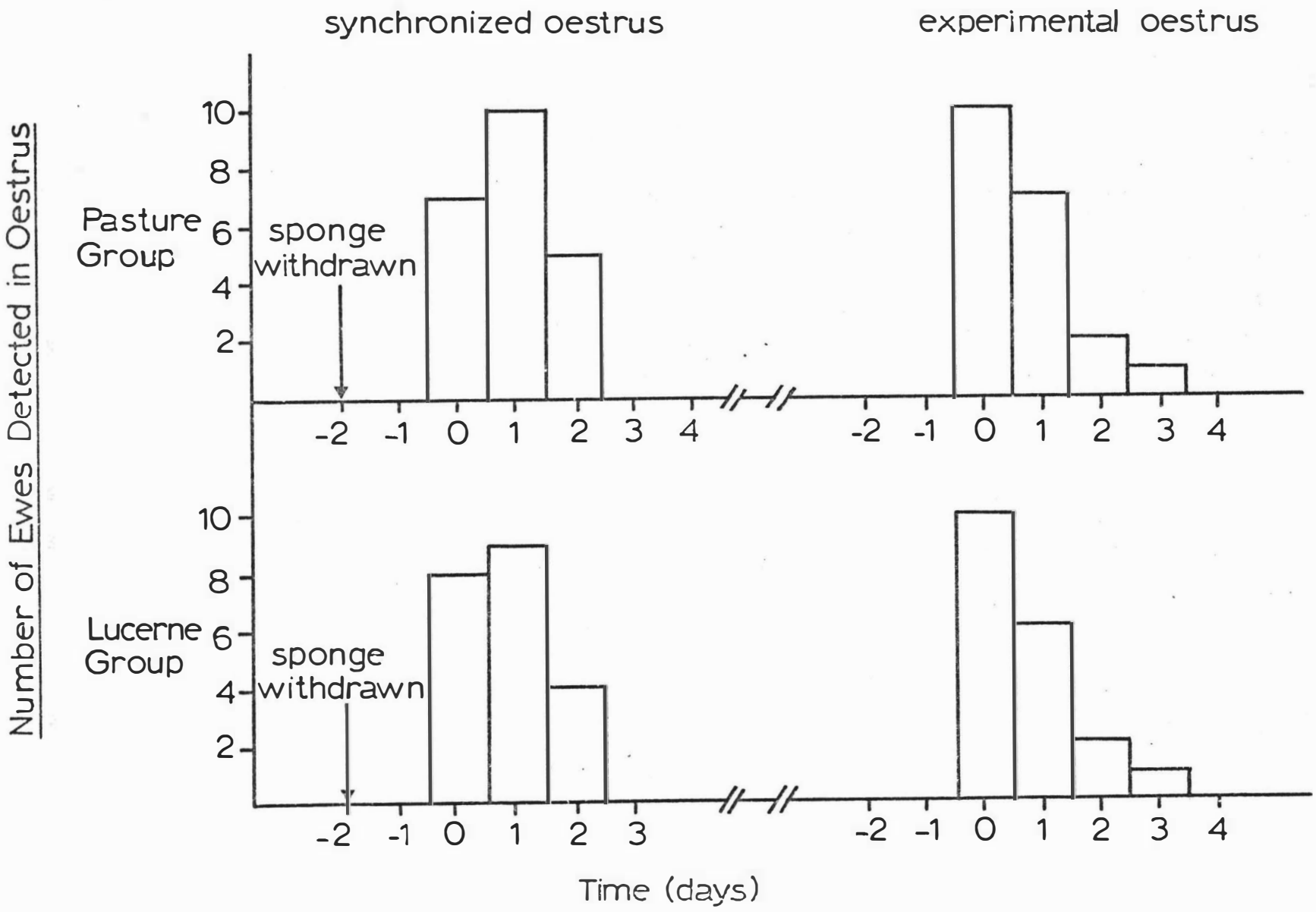


Fig. 3·2 DISTRIBUTION OF THE ONSET OF OESTRUS – Expt. II

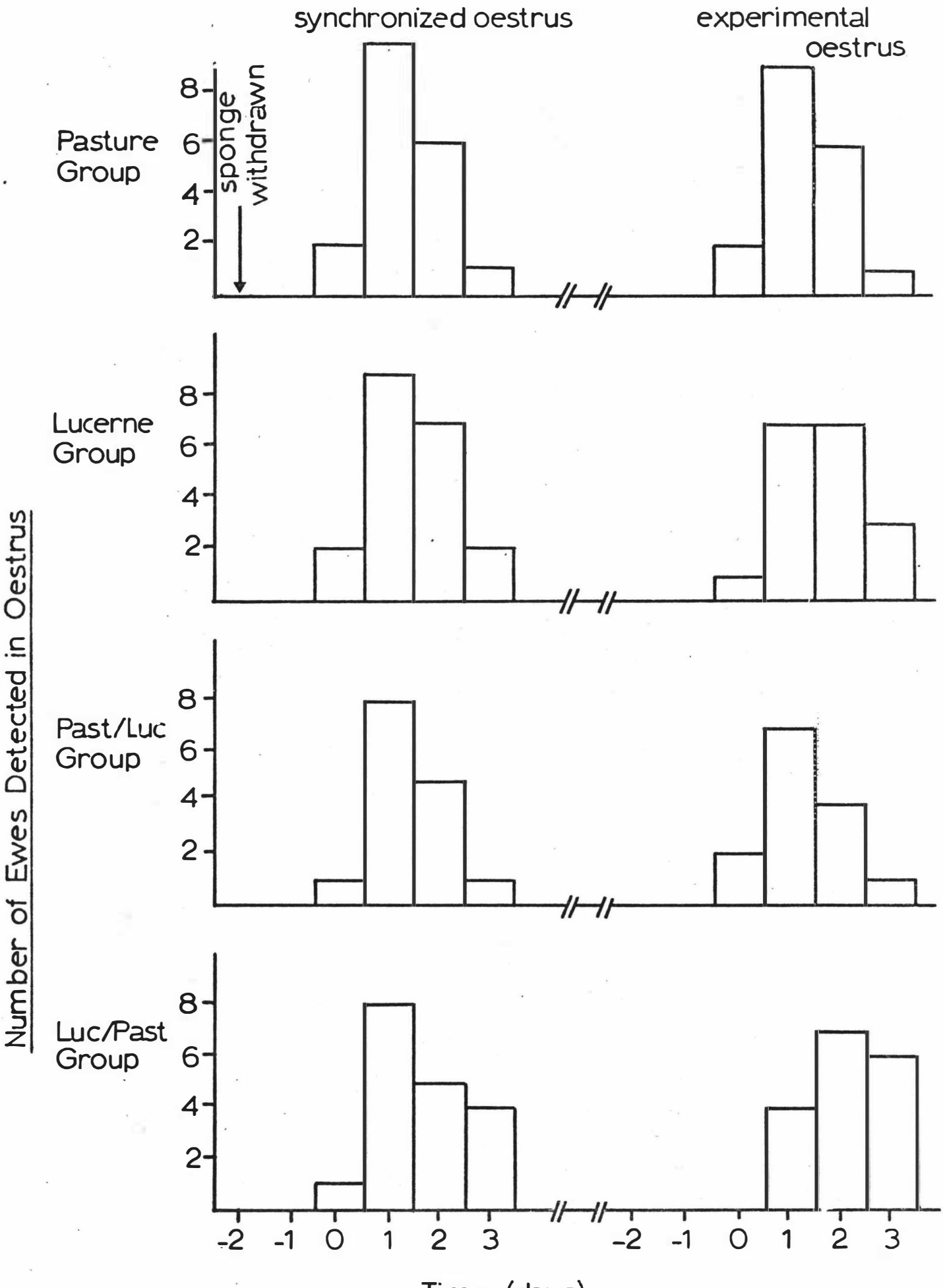
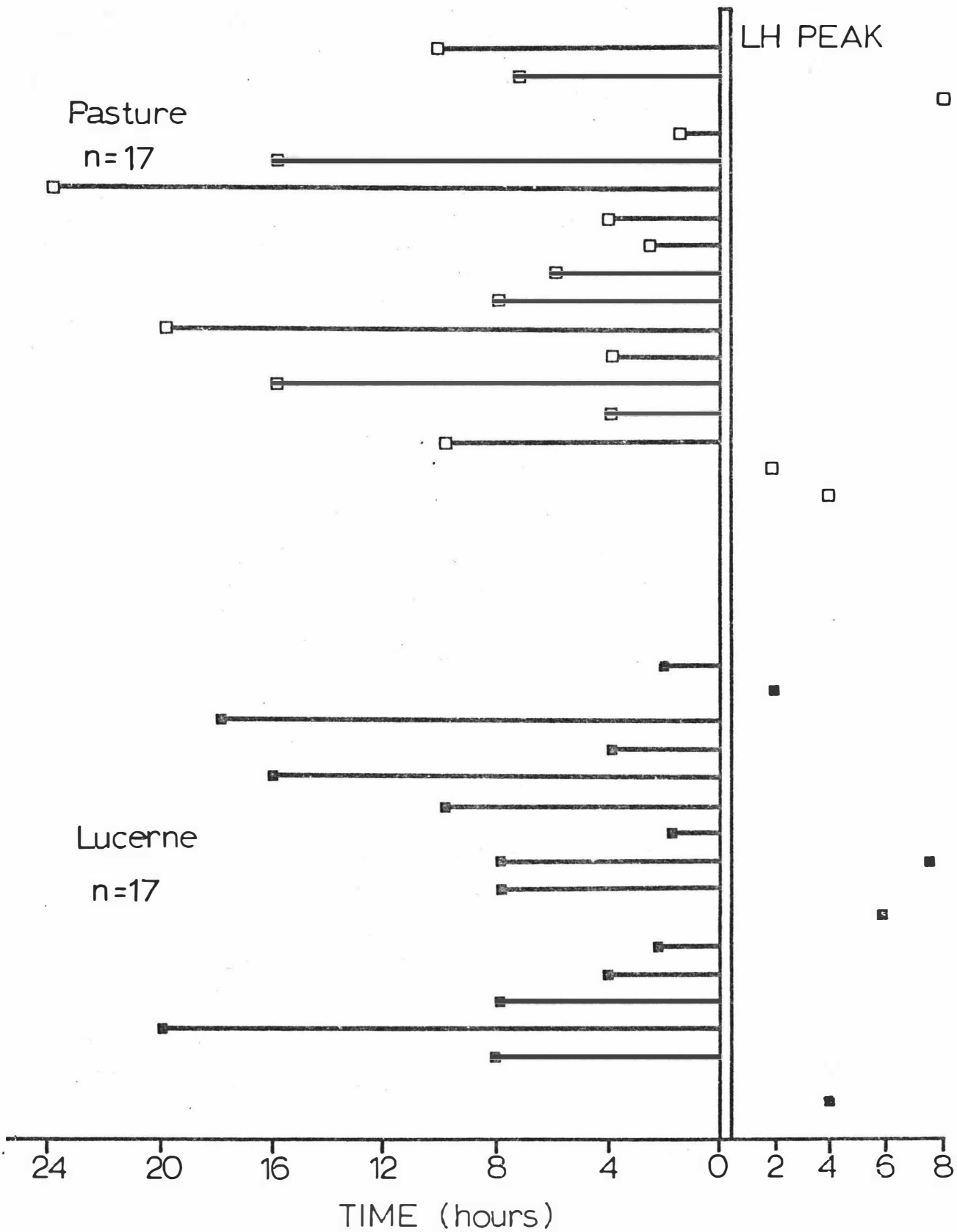


Fig. 3·3 ONSET OF OESTRUS - LH PEAK INTERVAL



be estimated.

(C) LUTEINIZING HORMONE SECRETION

(i) During the Oestrous Cycle

Mean daily concentrations of luteinizing hormone in peripheral plasma of ewes from both the pasture and the lucerne groups of Experiment I are listed in Table 3-4. Analyses of variance between the groups were made for each sample day. There were no significant differences in LH secretion at any stage of the oestrous cycle. Mean plasma LH concentrations ranged from 0.5 to 1.5 ng/ml over the cycle, excluding the pre-ovulatory peak. The patterns of secretion are shown in FIG. 3-4.

(ii) The Pre-Ovulatory LH Peak

Mean concentrations of luteinizing hormone in peripheral plasma, measured at 2-hourly intervals from the onset of oestrus, are shown in Table 3-5. The pre-ovulatory LH peaks were included in this period. Analyses of variance between the lucerne and the pasture groups, at each sample time, failed to show any significant differences. The pre-ovulatory LH secretion patterns are shown in FIG 3-5.

TABLE 3-4: MEAN PLASMA LH CONCENTRATIONS (ng/ml) DURING THE OESTROUS CYCLE IN EWES GRAZING PASTURE OR OESTROGENIC LUCERNE

	DAY-7	-6	-5	-4	-3	-2	-1	0	+1
PASTURE	0.559	0.452	0.435	0.445	0.7561	0.949	1.03	87.05	0.591
(Mean±SE)	±0.17	±0.12	±0.11	±0.13	±0.12	±0.26	±0.32	±3.92	±0.1
LUCERNE	0.4065	0.573	0.3071	0.409	0.573	1.247	1.161	91.14	1.04
Mean±SE)	±0.13	±0.14	±0.11	±0.13	±0.5	±0.32	±0.25	±6.55	±0.11

	DAY+2	+3	+4	+5	+6	+7	+8	+9	+10
PASTURE	0.9750	0.765	0.859	0.749	0.749	0.585	0.534	0.434	0.546
Mean±SE)	±0.36	±0.17	±0.2	±0.21	±0.19	±0.16	±0.14	±0.11	±0.16
LUCERNE	1.5648	1.037	1.041	0.571	0.399	0.673	0.408	0.433	0.556
(Mean±SE)	±0.41	±0.32	±0.29	±0.16	±0.12	±0.12	±0.11	±0.14	±0.13

(Day 0 of oestrous cycle corresponds to time of pre-ovulatory LH peak)

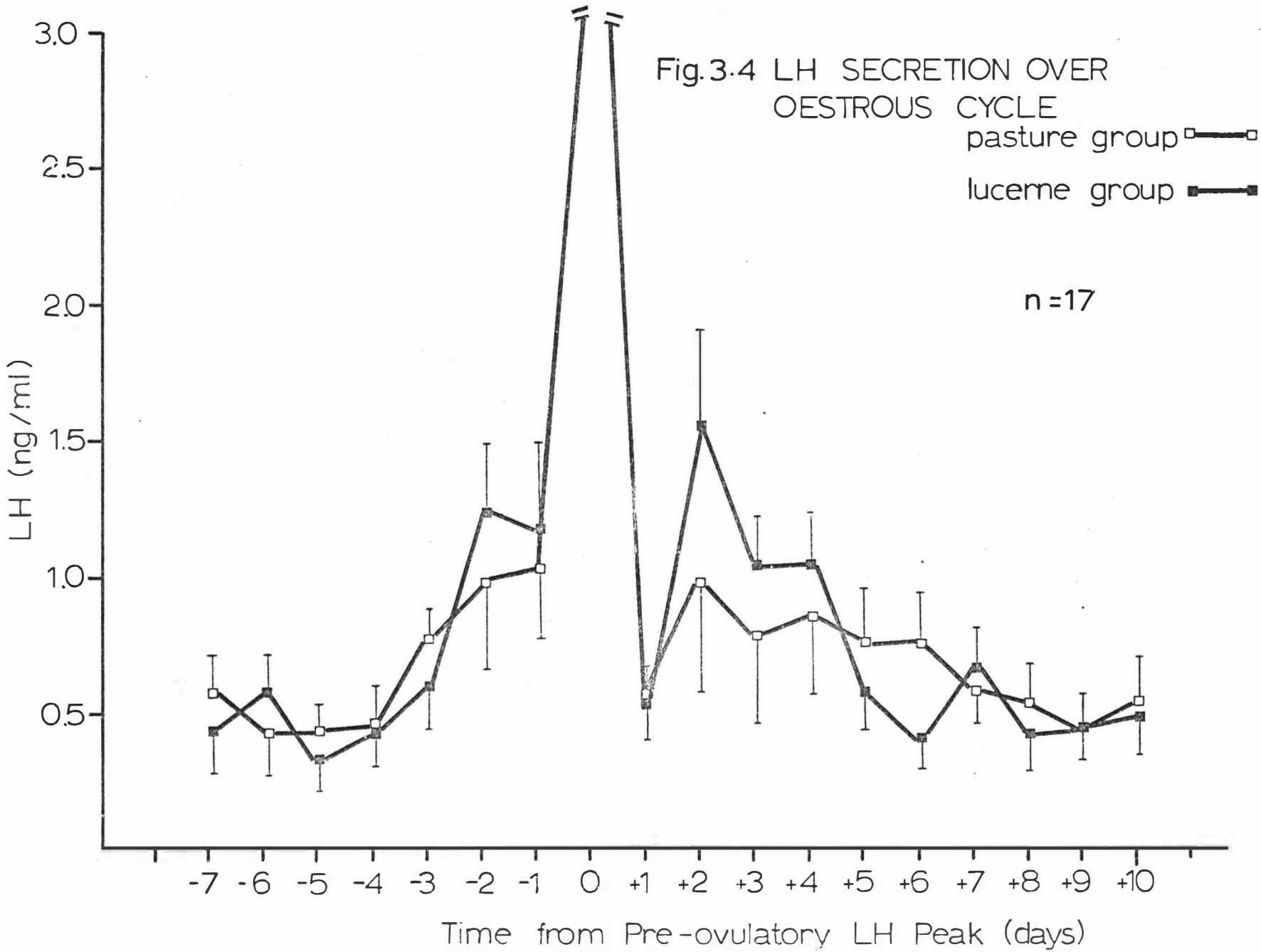


Fig. 3·4 LH SECRETION OVER OESTROUS CYCLE
pasture group
lucerne group
n = 17
LH (ng/ml)
3.0
2.5
2.0
1.5
1.0
0.5
-7 -6 -5 -4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8 +9 +10
Time from Pre-ovulatory LH Peak (days)

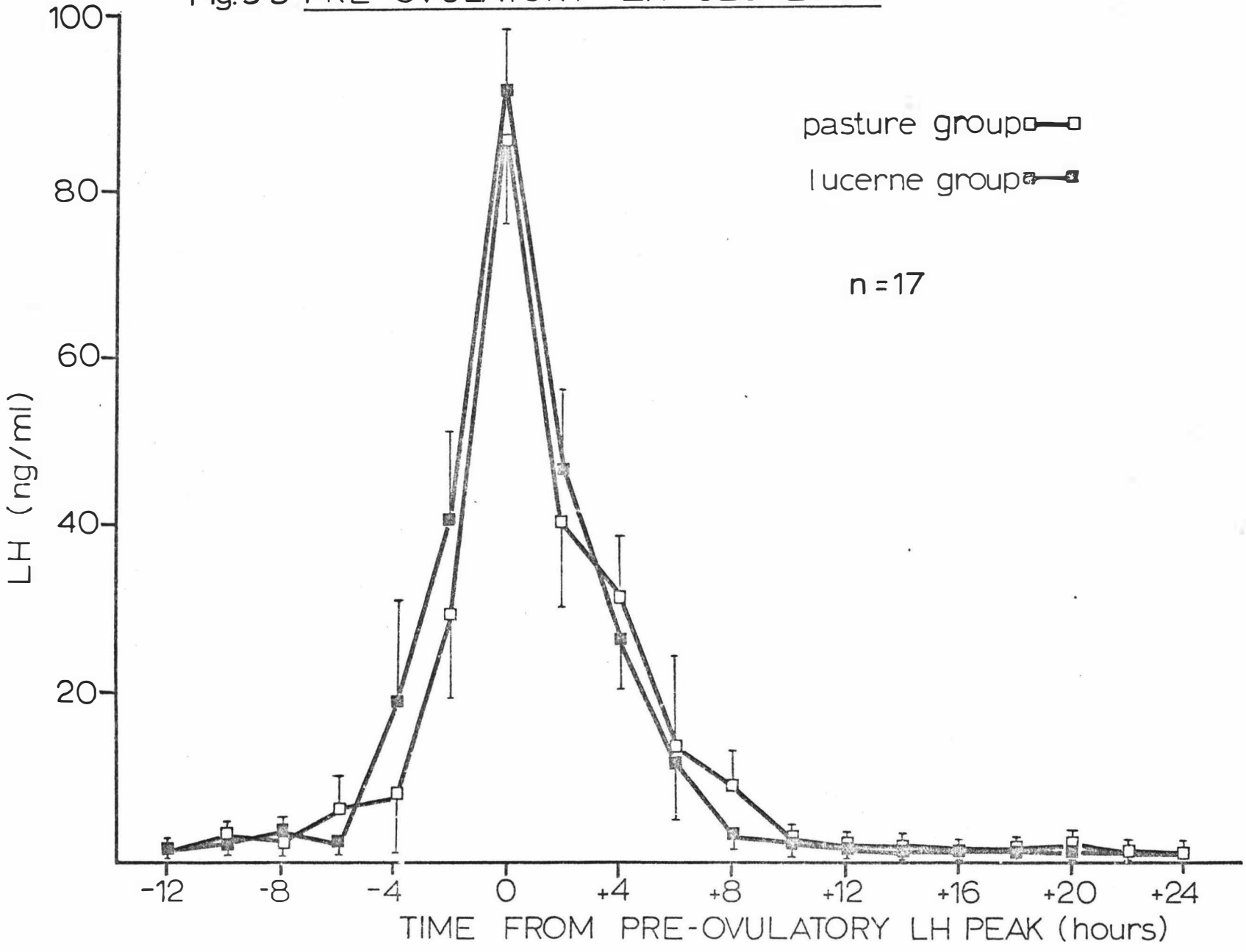
TABLE 3-5: PRE-OVULATORY PLASMA LH CONCENTRATIONS (ng/ml) IN EWES GRAZING PASTURE OR OESTROGENIC LUCERNE

	HOUR-12	-10	-8	-6	-4	-2	0	+2
PASTURE	1.13	2.43	1.85	6.04	7.99	29.20	87.05	40.26
	±0.37	±0.59	±0.35	±2.39	±2.54	±4.84	±3.92	5.82
LUCERNE	1.71	1.96	3.41	2.51	18.91	39.99	91.14	46.23
	±0.4	±0.67	±0.99	±0.48	±3.93	6.30	±6.55	±5.24

	+4	+6	+8	+10	+12	+14	+16	+18
PASTURE	31.38	13.37	8.68	2.70	1.40	0.84	0.75	0.90
	±4.11	±5.89	±2.68	±0.57	±0.26	±0.18	±0.19	±0.38
LUCERNE	26.19	11.63	3.24	2.40	1.11	0.83	0.72	0.51
	±3.74	±3.56	±0.37	±0.25	±0.28	±0.18	±0.20	±0.08

	+20	+22	+24
PASTURE	1.19	0.51	0.25
	±0.38	0.24	±0.06
LUCERNE	0.41	0.44	0.66
	±0.06	±0.09	±0.19

Fig. 3·5 PRE-OVULATORY LH SECRETION



(D) OVULATION RATES

(i) Experiment I

Mean ovulation rates for both the pasture and the lucerne groups are shown in Table 3-6. Mean ovulation rates for the subgroups within these treatment groups (Border Leicester x Romney ewes and Romney ewes) are also given. Oestrogenic lucerne depressed mean ovulation rates for all groups. The depression of ovulation rates was greater for ewes in the Border Leicester x Romney group (0.73 ovulations per ewe) than for ewes in the Romney group (0.59 ovulations per ewe).

TABLE 3-6: MEAN OVULATION RATES FOR EWES GRAZING PASTURE OR OESTROGENIC LUCERNE-EXPERIMENT I

	Pasture (Mean±SE)	Lucerne (Mean±SE)	Difference in Ovulation Rate Ovul^{n}/Ewe	Percent	Signif(χ^2)
Border L x Romney	2.50±0.12 (8)	1.77±0.22 (9)	0.73	29.2	3.666
Romney	2.09±0.16 (9)	1.50±0.15 (8)	0.59	28.3	3.216
Total	2.29±0.12 (17)	1.65±0.13 (17)	0.67	29.0	11.066***

Figure in perenthesis represents number of ewes in group.

(***ρ<0.001)

(ii) Experiment II

Mean ovulation rates for each of the four treatment groups are shown in Table 3-7. Oestrogenic lucerne depressed ovulation rates when compared to the control group (Pasture group), but only when it was ingested in the latter half of the oestrous cycle.

TABLE 3-7: MEAN OVULATION RATES FOR EWES GRAZING PASTURE OR OESTROGENIC LUCERNE-EXPERIMENT II

TREATMENT	OVULATION RATE (MEAN±SE)	n.
Pasture (P)	1.75 ± 0.13	16
Lucerne (L)	1.35 ± 0.12	17
Pasture-Lucerne (PL)	1.10 ± 0.10	10
Lucerne-Pasture (LP)	1.55 ± 0.12	18

Comparisons of the differences in ovulation rates between treatments are shown in Table 3-8.

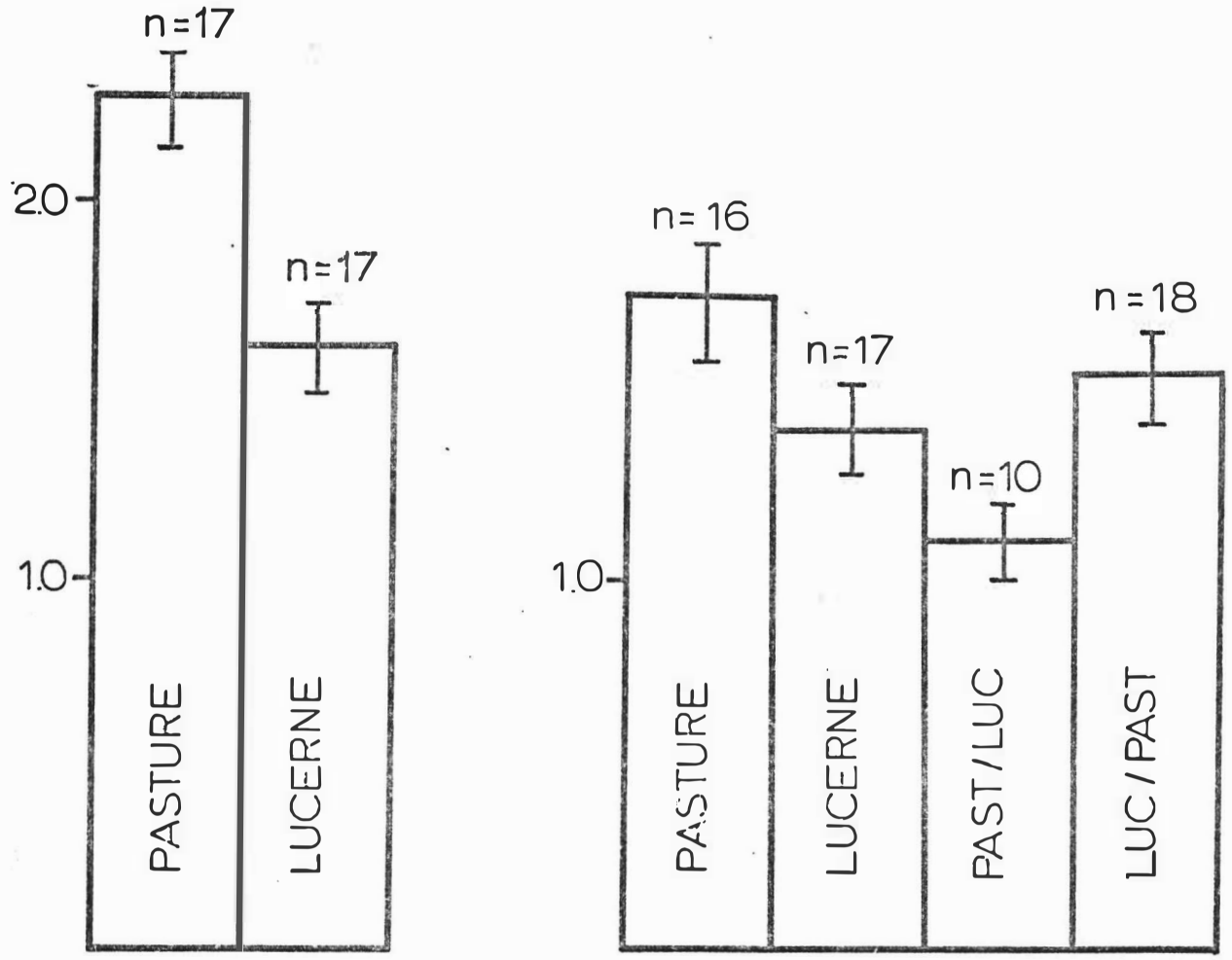
TABLE 3-8: COMPARISONS OF THE DIFFERENCES IN OVULATION RATES BETWEEN TREATMENTS - EXPERIMENT II

	Differences Ovulations/ Ewe	Percent Differences	χ^2 Statistic	Significance
P. v L.	0.40	22.9	3.3452	#
P. v PL.	0.65	37.1	8.5467	**
P. v LP.	0.20	11.4	0.6243	n.s.
L. v PL.	0.25	18.5	2.1096	n.s.
LP.v L.	0.20	12.9	1.9983	n.s.
LP.v PL.	0.40	29.0	5.5934	*

(n.s. = not significant * $\rho<0.05$ ** $\rho<0.01$ # $0.1>p>0.05$)

There were no significant differences in ovulation rates between the two groups which grazed pasture in the latter half of the cycle (Pasture and Lucerne-Pasture), or between the two groups which grazed lucerne in the latter half of the cycle (Lucerne and Pasture-Lucerne). However, there were differences in ovulation rate between the two treatment-interchange groups.

Fig. 3·6 OVULATION RATES



Expt. 1

Expt. 2

(iii) Corpora Lutea Diameter

The diameter of each corpus luteum was calculated as the mean of two measurements taken at right angles at the widest point. The mean corpus luteum diameter in ewes from each treatment group, are shown in Table 3-9. There were no significant differences between treatment groups.

TABLE 3-9: MEAN CORPORA LUTEA DIAMETER (mm) THREE DAYS POST-OESTRUS

PASTURE	LUCERNE	PASTURE-LUCERNE	LUCERNE-PASTURE
5.97 ± 0.37	5.59 ± 0.25	6.05 ± 0.38	5.85 ± 0.25

(E) FOLLICLE POPULATIONS

(i) Surface Follicles

In Experiment I, the number of unruptured follicles with a diameter greater than 3.5mm that were present on the surface of the ovaries, was recorded at laparotomy. This was combined with the number of corpora lutea present to give an estimate of the total number of large follicles present at the time of ovulation. From this 'estimate' of total follicle numbers, the ratio of the number of large follicles present per ovulation is calculated. These data are shown in Table 3-10.

In Experiment II, all unruptured surface follicles with a diameter of greater than 2mm were recorded on recovery of the ovaries at slaughter. Data from this experiment is shown in Table 3-11.

The removal of the ovaries in Experiment II enabled small follicles to be measured with more accuracy than could be achieved *in situ* in Experiment I.

TABLE 3-10: SURFACE FOLLICLES OF GREATER THAN 3.5mm DIAMETER, CORPORA LUTEA AND FOLLICLES PER OVULATION FOR EWES GRAZING PASTURE OR OESTROGENIC LUCERNE-EXPERIMENT I

	PASTURE				LUCERNE				Significance of Diff.
	n.	Follicles	C.L.	Est. Total	Foll/Ovn	n.	Follicles	C.L.	Est. Total	Foll/Ovn	Foll/Ovn
Border Leic. X Romney Ewes	8	1.80 ±0.49	2.50 ±0.17	4.30 ±0.58	1.70 ±0.17	9	2.55 ±0.77	1.77 ±0.22	4.33 ±0.69	2.89 ±0.73	(χ^2) 0.5396
Romney Ewes	9	2.09 ±0.65	2.09 ±0.16	4.18 ±0.72	1.95 ±0.32	8	1.66 ±0.53	1.50 ±0.15	3.17 ±0.53	2.25 ±0.44	0.8261
Total	17	1.95 ±0.68	2.29 ±0.12	4.24 ±0.46	1.83 ±0.18	17	2.05 ±0.44	1.62 ±0.13	3.67 ±0.44	2.52 ±0.40	5.1332*

(* $\rho<0.05$)

Est. Total (Estimated Total Follicle Numbers) - includes follicles plus corpora lutea and is an estimate of the numbers of follicles present at the instant of ovulation.

n. = Numbers of animals in each group.

TABLE 3-11: SURFACE FOLLICLES OF GREATER THAN 2.0mm DIAMETER, CORPORA LUTEA AND FOLLICLES PER OVULATION FOR EWES GRAZING PASTURE OF OESTROGENIC LUCERNE - EXPERIMENT II

PASTURE				LUCERNE			
Surf Follicle	Corpora Lutea	Estimated Total	Follicles/Ovn	Surf Follicle	Corpora Lutea	Estimated Total	Follicles/Ovn
5.08 ± 0.46	1.75 ± 0.13	6.83 ± 0.61	3.90 ± 0.32	4.51 ± 0.39	1.35 ± 0.12	5.86 ± 0.53	4.34 ± 0.42
(n = 16)				(n = 17)			

PASTURE-LUCERNE				LUCERNE-PASTURE			
Surf Follicle	Corpora Lutea	Estimated Total	Follicles/Ovn	Surf Follicle	Corpora Lutea	Estimated Total	Follicles/Ovn
4.07 ± 0.43	1.1 ± 0.1	5.17 ± 0.58	4.70 ± 0.52	3.81 ± 0.31	1.55 ± 0.12	5.36 ± 0.55	3.46 ± 0.39
(n = 10)				(n = 18)			

n = Number of animals in each group.

(ii) Total Follicle Populations

The total numbers of follicles present, with a diameter greater than 2mm, were recorded after dissection of the ovaries. There were no significant differences, nor any apparent trends, evident in total follicle populations between treatments (Table 3-12).

Estimates of the total numbers of follicles present at the instant of ovulation were made, using the same procedure as was used in surface follicle estimates. Similarly, the total number of follicles present per ovulation was calculated. These data are shown in Table 3-13 for follicles with a diameter of greater than 3.5mm, and in Table 3-14 for follicles with a diameter of greater than 2.0mm.

Total follicle populations are shown in FIG.3-7.

There were no significant differences between treatment groups, in the mean number of follicles of a diameter greater than 2.0mm produced per gram of ovarian tissue (Table 3-15).

TABLE 3-15: FOLLICLES OF GREATER THAN 2.0mm DIAMETER PER GRAM OF OVARIAN TISSUE

PASTURE	LUCERNE	PASTURE-LUCERNE	LUCERNE-PASTURE
1.93 ± 0.31	1.84 ± 0.26	2.23 ± 0.34	2.02 ± 0.29

(F) OVARIAN AND REPRODUCTIVE TRACT RESPONSE

(i) Ovarian and Uterine Weights

Mean ovarian and uterine weights for each treatment group are shown in Table 3-16. There were no significant differences in organ weights between groups.

TABLE 3-12: OVARIAN FOLLICLE POPULATIONS OF EWES GRAZING PASTURE OR OESTROGENIC LUCERNE - EXPERIMENT II

(MEAN ± SE)

PASTURE					LUCERNE				
Follicles 2-3.5mm	Follicles 3.6-5.0	Follicles >5mm	Total No. Follicles	Follicles Per Gram Ovary	Follicles 2-3.5mm	Follicles 3.6-5.0	Follicles >5mm	Total No. Follicles	Follicles Per Gram Ovary
3.25±0.48	1.88±0.41	0.12±0.09	5.25±0.76	1.93±0.31	2.35±0.30	2.29±0.34	0.12±0.09	4.76±0.77	1.84±0.26
(n = 16)					(n = 17)				

PASTURE-LUCERNE					LUCERNE-PASTURE				
Follicles 2-3.5mm	Follicles 3.6-5.0	Follicles >5mm	Total No. Follicles	Follicles Per Gram Ovary	Follicles 2-3.5mm	Follicles 3.6-5.0	Follicles >5mm	Total No. Follicles	Follicles Per Gram Ovary
3.2 ±0.47	2.00±0.33	0.20±0.13	5.40±0.86	2.23±0.34	2.33±0.39	2.11±0.33	0.67±0.21	5.11±0.81	2.02±0.29
(n = 10)					(n = 18)				

n = Number of animals in each group.

TABLE 3-13: TOTAL NUMBER OF FOLLICLES OF GREATER THAN 3.5mm DIAMETER, CORPORA LUTEA AND FOLLICLES PER OVULATION - EXPERIMENT II

PASTURE				LUCERNE			
Follicles	Corpora Lutea	Estimated Total	Follicles/Ovn	Follicles	Corpora Lutea	Estimated Total	Follicles/Ovn
2.00±0.48	1.75±0.13	3.75±0.49	2.14±0.23	2.41±0.46	1.35±0.12	3.76±0.37	2.78±0.30
(n = 16)				(n = 17)			

PASTURE-LUCERNE				LUCERNE-PASTURE			
Follicles	Corpora Lutea	Estimated Total	Follicles/Ovn	Follicles	Corpora Lutea	Estimated Total	Follicles/Ovn
2.20±0.46	1.10±0.09	3.30±0.40	3.00±0.26	2.78±0.31	1.55±0.12	4.33±0.38	2.79±0.31
(n = 10)				(n = 18)			

TABLE 3-14: TOTAL NUMBER OF FOLLICLES OF GREATER THAN 2.0mm DIAMETER, CORPORA LUTEA AND FOLLICLES PER OVULATION - EXPERIMENT II

PASTURE				LUCERNE			
Follicles	Corpora Lutea	Estimated Total	Follicles/Ovn	Follicles	Corpora Lutea	Estimated Total	Follicles/Ovn
5.25±0.61	1.75±0.13	7.00±0.80	4.00±0.45	4.76±0.61	1.35±0.12	6.11±0.55	4.52±0.45
(n = 16)				(n = 17)			

PASTURE-LUCERNE				LUCERNE-PASTURE			
Follicles	Corpora Lutea	Estimated Total	Follicles/Ovn	Follicles	Corpora Lutea	Estimated Total	Follicles/Ovn
5.40±0.63	1.10±0.10	6.50±0.45	5.91±0.48	5.11±0.59	1.55±0.12	6.66±0.62	3.98±0.39
(n = 10)				(n = 18)			

n = Number of animals in each group.

Fig. 3·7 POST-OVULATION FOLLICLE POPULATIONS

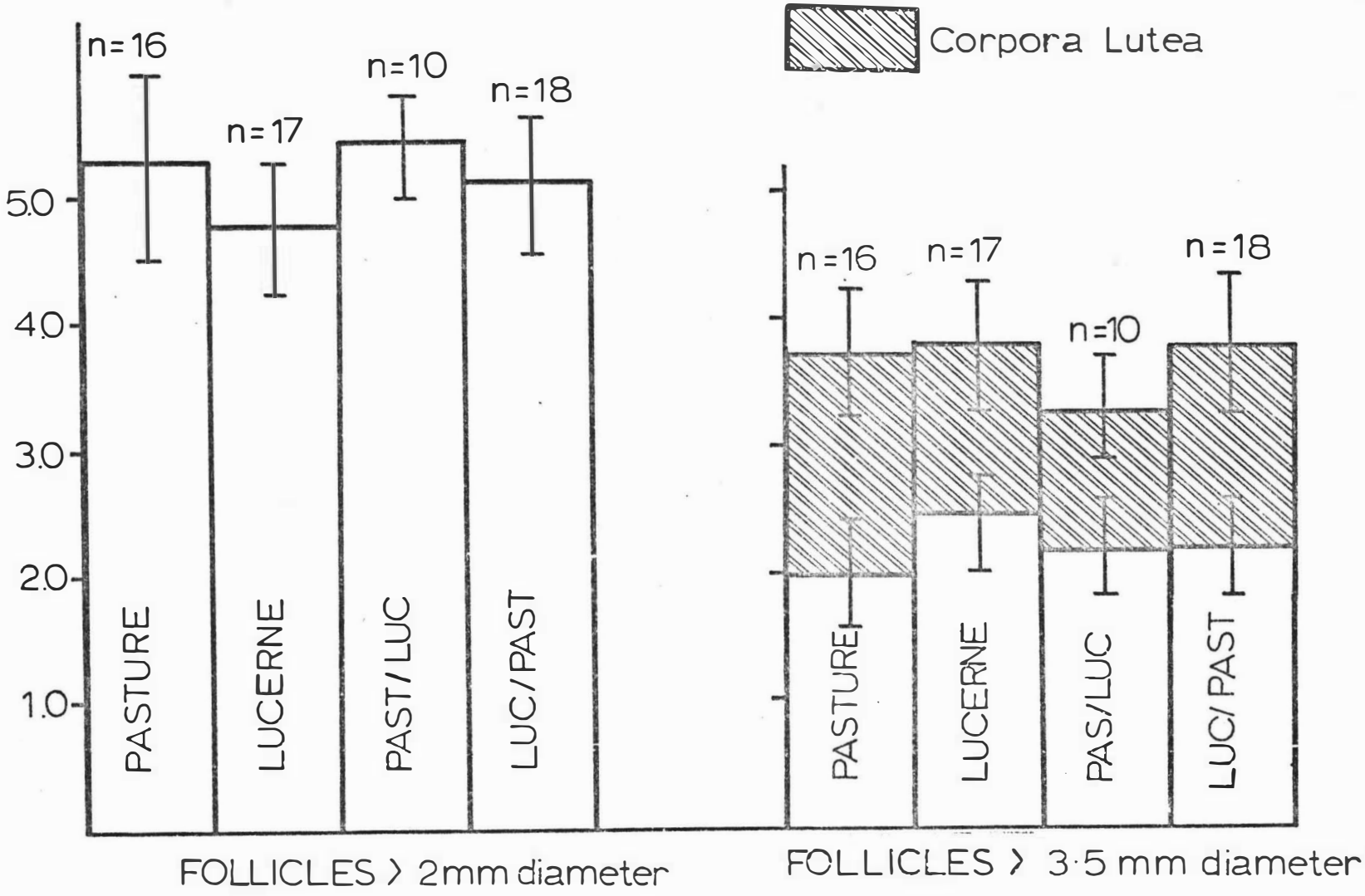


TABLE 3-16: OVARIAN AND UTERINE WEIGHTS (g) IN EWES GRAZING PASTURE OR OESTROGENIC LUCERNE

	PASTURE	LUCERNE	PASTURE-LUC	LUCERNE-PAST
Ovarian Weight (Mean ± SE)	2.72 ± 0.16	2.58 ± 0.14	2.42 ± 0.19	2.52 ± 0.12
Uterine Weight (Mean ± SE)	59.06 ± 2.64	63.72 ± 1.90	60.38 ± 4.51	67.74 ± 2.75

(ii) Reproductive Tract Histology

There were no significant differences between groups in the height of epithelial cells, in any of the tract tissue examined. Mean epithelial cell heights are shown in Table 3-17.

TABLE 3-17: EPITHELIAL CELL HEIGHT (μ) OF REPRODUCTIVE TRACT TISSUE FROM EWES GRAZING PASTURE OR OESTROGENIC LUCERNE

TISSUE	PASTURE	LUCERNE	PASTURE-LUC	LUCERNE-PAST
Vagina	92.76 ± 8.54	79.13 ± 5.69	99.88 ± 4.19	94.92 ± 7.79
Cervix	26.09 ± 1.09	24.86 ± 1.11	25.1 ± 1.25	26.21 ± 0.83
Uterus	27.35 ± 0.98	27.84 ± 0.78	25.62 ± 1.00	25.65 ± 0.99
Fallopian Tube	24.49 ± 0.89	24.58 ± 0.57	24.43 ± 0.96	23.01 ± 0.99

(G) SUMMARY

1. The coumestan levels in the lucerne were moderately high and the daily intake of the ewes would have easily exceeded that required to induce known oestrogenic responses.

2. Oestrogenic lucerne did not effect progestagen-induced synchronization of oestrus, or the mean length of oestrous cycles.

3. The length of the interval from the onset of oestrus to the pre-ovulatory surge of LH varied markedly between animals. The coumestans had no detectable influence.

4. The measurement of the chloride ion content of cervical mucus could not be utilized to determine the time of ovulation. Consequently the length of the interval from the LH peak to ovulation could not be determined.

5. The ingestion of coumestans did not significantly alter the secretion of luteinizing hormone. Secretion patterns throughout the cycle and over the pre-ovulatory LH surge were unchanged.

6. Oestrogenic lucerne depressed ovulation rates by up to 29 percent, but only when lucerne was consumed for the latter half of the oestrous cycle, was the depression of ovulation rate statistically significant.

7. The higher the 'natural' ovulation rate of ewes, the greater is the coumestan-induced depression of ovulation rate.

8. The numbers of follicles present in the ovaries were unchanged, although the number of follicles that ovulated was lowered by the ingestion of coumestans.

9. The mean diameter of corpora lutea was not affected by the ingestion of coumestans.

10. The mean uterine weights and ovarian weights of ewes grazing oestrogenic lucerne, did not differ significantly from those of ewes grazing non-oestrogenic ryegrass/clover pasture, when compared three days post-oestrus.

11. The mean height of epithelial cells in genital tract tissue, did not differ between ewes grazing oestrogenic lucerne and ewes grazing non-oestrogenic ryegrass/clover pasture, when compared three days post-oestrus.

C H A P T E R I V

DISCUSSION

CHAPTER IV

DISCUSSION

(A) OESTROGENIC LUCERNE

Severe infection of lucerne leaves by the fungi Pseudopeziza medicaginis and Leptosphaerulina briosiana (Loper and Hansen, 1964), both of which are common fungal pathogens of lucerne in New Zealand (Close, 1967), produced material of high coumestan content. A similar degree of oestrogenic potency was apparent under similar conditions with this lucerne stand in a previous trial (McCutcheon, 1976).

The estimates of daily intake of coumestans suggest that plasma coumestan concentrations would be well in excess of the levels necessary to induce oestrogenic responses (Shutt et al., 1969). A recent trial (Smith et al., unpublished data) has shown that as little as 25ppm coumestan in the feedstuff is sufficient to suppress ovulation rate, and the degree of suppression is increased by an increase in coumestan content.

(B) OESTRUS PHENOMENA

(i) Oestrus Synchronization

Progestagen treatment resulted in a high proportion of ewes showing oestrus over a four day period, following sponge withdrawal. Some ewes were incapable of responding to progestagen treatment, owing to having blind tracts or pregnant uteri, or to losing sponges during synchronization. If these animals are disregarded, then 93 percent of ewes in Experiment I and 91 percent of ewes in Experiment II were detected in heat over the synchronized oestrus. A high degree of

synchrony was maintained at the following oestrus, with 82 percent (Experiment I) and 85 percent (Experiment II) of the ewes in oestrus over a comparable four day period. The higher proportion of ewes undetected in the second oestrous cycle in Experiment I, may reflect an inadequacy in the period of oestrus detection, rather than any biological phenomenon. The skewed distribution of the onset of oestrus (FIG. 3-1) suggests that some ewes may have been detected if rams were introduced earlier.

Oestrogenic lucerne did not affect the degree of oestrus synchronization attained, or the incidence of 'silent heats', although behavioural oestrus has been related to plasma oestrogen levels (Piper and Foote, 1968; Goding et al., 1970; Scaramuzzi et al., 1971b). Conversely treatment of cycling ewes with antisera to endogenous oestrogens prevents the occurrence of behavioural oestrus (Scaramuzzi, 1975; Fairclough et al., 1976), but the administration of anti-sera to phyto-oestrogens does not prevent oestrus (Cox and Wilson, 1976).

(ii) The Oestrus-Ovulation Interval

The wide variation in the length of the interval from the onset of oestrus to the pre-ovulatory LH peak, between individual ewes, is in agreement with observations by Lamming, (pers. com. 1978). Lamming suggested that this is due to biological variation rather than to inadequate oestrus detection. Such wide variation between individuals would mask any differences induced by lucerne.

The administration of exogenous steroid oestrogens increases oestrus duration (Scaramuzzi et al., 1971a; Land et al., 1972). There is a linear relationship between oestrogen dose and the duration of the induced oestrus, however, ewes exhibit a refractoriness to repeated administration of oestrogen (Scaramuzzi et al., 1972). Land et al., (1972) suggested that highly fecund breeds were more sensitive to exogenous oestrogens in

relation to this oestrus response. There is no evidence to suggest that coumestans alter the duration of oestrus.

The failure of the cervical mucus chloride test in estimating the time of ovulation may have been the result of poor sampling techniques. The erratic changes in chloride concentrations may have been due to variation in the quantity of mucus collected at each sampling. Turnbull et al., (1967) and Lindsay & Francis (1969) suggested that chloride concentrations of cervical mucus may be utilized as an assay for phyto-oestroge intake in ewes. In this trial there were no differences detected in mucus chloride content, between ewes in the lucerne and pasture groups.

In conclusion, these experiments showed that there was considerable variation in the length of the interval from the onset of oestrus to the pre-ovulatory LH peak. We were unable to determine the length of the interval from the pre-ovulatory LH peak to ovulation. However Cumming et al., (1973) found a high degree of constancy in the length of the interval from LH peak to ovulation, in ewes of widely differing ages and under a number of treatments. This would suggest that there is considerable variation between animals in the interval from the onset of oestrus to the pre-ovulatory LH peak.

(C) LUTEINIZING HORMONE SECRETION

The depression in levels of receptor-bound endogenous oestrogens of ewes grazing lucerne (Newsome and Kitts, 1977) suggests that total endogenous oestrogen concentrations may also be depressed. A relationship between steroid oestrogens and luteinizing hormone secretion has clearly been established.

Ovariectomy, and the subsequent fall in plasma oestrogen levels, results in an elevation of basal LH levels (Reeves, et al., 1972; Diekman and Malvern, 1973). The administrationof exogenous oestrogens to ovariectomized ewes induces a biphasic response; an initial depression in LH concentrations, followed by a large elevation similar to pre-ovulatory levels (Radford et al., 1969; Goding et al., 1970; Howland and Palmer, 1973). Feeding oestrogenic

clover, of high isoflavone content, to ovariectomized ewes mimics the administration of exogenous steroid oestrogens on LH secretion (Hearnshaw et al., 1977).

In the present trials there were no increases in basal LH levels in ewes grazing oestrogenic lucerne. Presumably, the depression of endogenous oestrogen levels, if it did occur, was not of sufficient magnitude to affect LH secretion, or alternatively, the coumestans compensated for lowered endogenous oestrogen levels.

If the coumestans do lower endogenous oestrogen levels, it is feasible that they may affect the pre-ovulatory LH surge as this is primarily a response to high levels of circulating oestrogens of the previous day (Moore et al., 1969; Cox et al., 1971). Oestradiol injections on days 3-4 (Bolt et al., 1971) or on days 10-12 (Howland et al., 1971) of the oestrous cycle, will induce 'extra' pre-ovulatory type LH surges in cyclic ewes. In the present trial such extra pre-ovulatory LH peaks were not induced by the ingestion of coumestans, nor was the 'true' pre-ovulatory LH peak affected.

The LH profiles from ewes grazing oestrogenic lucerne in this trial, failed to demonstrate changes in either the negative-feedback effects of oestrogens on basal LH levels, or their positive-feedback effects on pre-ovulatory LH surges. It is possible that either or both of these oestrogenic effects did occur, but were not apparent. To obtain accurate quantitative secretion patterns, the interval between sampling must be less than the half-life of the hormone (LH ½ life is approximately 20 minutes). Sampling at such frequent intervals would demand the use of indwelling catheters and would also restrict the numbers of animals used.

With the numbers of animals involved in this trial, any major changes in LH secretion patterns should have been detected with 2-hourly

sampling. Therefore it is concluded that if oestrogenic lucerne did affect secretion patterns of LH, the changes were not extensive.

(D) DEPRESSION OF OVULATION RATES

Ewe liveweights were recorded prior to, during and at the completion of the experiments. There were no significant differences in ewe liveweights between groups at any of these times, which would indicate the absence of a 'body condition' effect on ovulation rate comparisons. Similarly the random assignment of ewes to each treatment group should have minimised any genetic differences in ovulation rates between groups. Therefore it may be assumed that any major variations in ovulation rates were due to the treatment - the ingestion of oestrogenic lucerne.

In both experiments the ingestion of oestrogenic lucerne depressed ovulation rates. This is in agreement with the observations of other workers (Scales and Moss, 1976; McCutcheon, 1976; Scales, et al., 1977). The marginal levels of significance between groups reflects the difficulty in analysing discrete data (number of ovulations) with relatively small numbers of animals. This is clearly evident from Experiment I, when the depression of ovulation rate within the breed subgroups (Romney and Border Leicester x Romney) is considered. Although the degree to which ovulation rates were depressed in these groups (29.2 percent and 28.3 percent respectively) is comparable to the depression of ovulation rate when all ewes in Experiment I are considered (29 percent) it is not statistically significant. However, when all ewes are considered, this same degree of ovulation rate depression is highly significant ($p<0.001$).

Those ewes of higher fecundity involved in these trials, were affected most by the ingestion of coumestans. In Experiment I, mean ovulation rate of Border Leicester x Romney ewes is depressed by 0.73 ovulations per ewe compared to 0.5 ovulations per ewe for Romney ewes. In a similar manner, the ovulation rate of the control animals (Pasture group) was depressed unequally between Experiment I and Experiment II. The control ewes of Experiment I had a higher inherent ovulation rate than those of Experiment II (2.29 ovulations per ewe compared to 1.75 ovulations per ewe), and they were depressed to a greater degree by oestrogenic lucerne (29 percent compared to 22 percent). This greater sensitivity of highly fecund ewes to the coumestans was also reported in earlier trials (Scales and Moss, 1976; Scales et al., 1977).

This breed variation in sensitivity to coumestans would appear to correspond to observed breed differences in sensitivity to exogenous steroid oestrogens. The administration of exogenous steroid oestrogens to cycling ewes increases the duration of oestrus (Land, 1970) and breeds of higher fecundity display a greater response than do the less prolific breeds (Land et al., 1972). Ewes of high fecundity are however, less sensitive to exogenous oestrogen-induced LH secretions (Land et al., 1976). An apparent contradiction to the higher sensitivity to coumestans in the depression of ovulation rate, is the report of Land et al. (1976) of a lower sensitivity to the exogenous oestradiol-induced depression of ovulation rate.

From the data collected in Experiment II, it is clearly evident that it is the ingestion of coumestans in the latter half of the oestrous cycle that is involved in ovulation rate depression. The grazing of oestrogenic lucerne early inthe oestrous cycle had no effect on ovulation rates provided that the ewes grazed non-oestrogenic pasture

in the latter half of the cycle. These findings are consistent with the claim that the number of follicles which are destined to ovulate, is determined within the three days prior to oestrus (Land, 1973; Findlay and Cumming, 1977; Bherer et al., 1977).

Hemicastration of ewes prior to day 14 of the oestrous cycle, leads to a transient increase in FSH levels coinciding with falling oestradiol levels, and increased follicular growth in the remaining ovary over the final three days of the cycle (Ramirez and Sawyer, 1974; Findlay and Cumming, 1977). In experiments where the feeding of lupin grain to ewes increased ovulation rates, increases in FSH levels were also reported (Brien et al., 1976). This strong, although indirect, correlation between high FSH levels and increased ovulation rates, prompts the suggestion that the converse situation occurs in ewes grazing lucerne. FSH levels in ewes ingesting coumestans may be depressed due to the intensification of the negative-feedback influence of oestrogens on gonadotrophin release.

(E) FOLLICLE POPULATIONS

It has previously been shown that breeds with a high inherent ovulation rate, ovulate a higher proportion of the Graafian follicles that develop (Bradford et al., 1971; Turnbull et al., 1977b). This characteristic is evident in the follicle population data of Experiment I. When all corpora lutea and unruptured follicles with a diameter of greater than 3.5mm are included in an estimate of the large follicle population, trends between the number of follicles and ovulation rate are apparent. Ewes of the Border Lecister x Romney subgroup had fewer follicles per ovulation than did ewes from the Romney subgroup. The low numbers of ewes involved probably precludes these differences from being statistically significant.

Oestrogenic lucerne increased the number of follicles present per follicle ovulating in the ewes of high fecundity, a trend which paralleled the correlation between high fecundity and ovulation rate depression.

In Experiment II, all surface follicles of a diameter greater than 2mm were incorporated in estimates of follicle populations, and similar trends were shown. The ingestion of oestrogenic lucerne in the latter half of the oestrous cycle increased the number of follicles present per ovulation. Exposure to coumestans in the early stages of the cycle did not affect follicle numbers. Total follicle populations, recorded after slicing the ovaries, showed trends similar to those shown by surface follicle populations.

It would appear that if the coumestans do suppress the maturation of follicles, then this suppression does not restrict increases in follicle size. Although follicles develop to a comparable size in ovaries of sheep grazing lucerne, there is some physiological restriction which allows only the most advanced follicles to develop the potential to ovulate. Although there is no direct evidence, a deficiency of FSH at this stage of follicular development would appear to be a possible cause of ovulation rate depression. The hemicastration experiments of Ramirez and Sawyer (1974) and Findlay and Cumming (1977) give strong indirect evidence of the requirement for FSH. Furthermore, reports of the properties of exogenous oestrogens in altering pituitary responsiveness, could account for suppression of FSH levels by the coumestans. The administration of oestradiol to ewes a few hours (4-6) prior to Gn-RH, depresses the gonadotrophin response (Pelletier and Signoret, 1970; Libertum et al., 1974). It would also appear that FSH is much more sensitive to this inhibitory effect of oestrogen than is LH (Salamonsen et al.,1973; Baird and Scaramuzzi, 1976; Findlay and Cumming, 1977).

It has been established, that in sheep those follicles destined to ovulate would have a diameter of greater than 0.5mm by day 6-7 of the oestrous cycle (Turnbull et al., 1977a). As the ingestion of oestrogenic lucerne in the first half of the cycle (up to day 9) does not influence ovulation rates, the 'initiation' of follicular growth is apparently unimportant in ovulation rate depression. It has been suggested that large follicles release a substance that suppresses follicle initiation (Peters et al., 1973). As a consequence of lowered ovulation rate, ewes grazing lucerne will have a greater number of large follicles present early in the next oestrous cycle. However, even if there is a depression in the numbers of follicles 'initiated' it is unlikely that this would be a factor limiting the determination of ovulation rates.

There were no significant differences between groups in mean ovarian weights nor in the number of follicles present per gram of ovarian tissue. Although these are limited observations, this would suggest that the coumestans do not have any major effects on ovarian metabolism that result in weight increases.

(F) PROGESTERONE SECRETION

Progesterone may influence follicular growth independently of its effects via the hypothalomo-pituitary axis. Follicular growth is more rapid in ovaries that contain a corpus luteum (Dufour et al., 1971b). Injection of progesterone into one ovary of anoestrous ewes, together with systemic administration of PMSG, results in more rapid follicular growth and in a greater number of follicles and corpora lutea in the injected than in the control ovary (Harned and Casida, 1971). This treatment does not alter oestrogen secretion (Rexroad and Casida, 1977).

Smith and Robinson (1969) found the diameter of a corpus luteum (an estimate of its mass) to be highly correlated to progesterone concentration in the ovarian vein blood plasma (an estimate of luteal function). The ingestion of coumestans did not influence luteal function as estimated by corpora lutea size.

(G) COUMESTANS AND THE REPRODUCTIVE TRACT

The ingestion of coumestans by overiectomized ewes results in the expression of the 'classical' oestrogenic characteristics in the reproductive tract. The administration of 7.5 to 25mg of coumestrol per day, induces maximal stimulation of uterine growth (Braden et al., 1967; Shutt et al., 1969; Kelly et al., 1976b). The coumestans also induce cornification of reproductive tract tissue in ovariectomized ewes, similar to that occurring at oestrus in intact ewes (Braden et al., 1967). Similarly, the ingestion of oestrogenic lucerne causes the secretion of cervical mucus, comparable to levels found at oestrus, in ovariectomized (Kelly, 1972; Kelly et al., 1976b) or in intact anoestrous ewes (Kelly et al., 1976_a). Synthetic coumestans have similar effects (Shutt et al., 1969).

In Experiment II reproductive tracts were examined three days post-oestrus, when those oestrogenic changes which occur at oestrus would still have been evident. There were no significant differences between ewes grazing pasture and ewes grazing oestrogenic lucerne, in the measurement of those oestrogenic traits examined - i.e. uterine weights and epithelial cell heights of reproductive tract tissue. Apparently the levels of endogenous oestrogens present at oestrus masked the effects of the ingested coumestans.

It has been demonstrated that the coumestans compete with oestradiol for oestrogen-receptors (Shemesh et al., 1972). It would

appear that, having bound to oestrogen receptors, the coumestan-receptor complex is capable of fully expressing those oestrogenic changes induced by the oestradiol-receptor complex, at least within the reproductive tract. Newsome and Kitts (1977) recently found that there were reduced levels of oestradiol-receptor binding in ewes grazing oestrogenic lucerne, although oestrogenic parameters of the reproductive tract are unchanged.

(H) CONCLUSIONS

The levels of coumestan intake by ewes grazing lucerne in these experiments were sufficient to induce all of its oestrogenic effects. These oestrogenic changes - increased uterine weight, increased cervical mucus production and cornification of reproductive tract tissue - would be readily expressed in ovariectomized ewes subjected to similar levels of coumestans. However, in intact cycling ewes these parameters did not differ from those shown by ewes grazing non-oestrogenic ryegrass/clover pasture. The single characteristic of the reproductive system which was consistently affected by oestrogenic lucerne, was a reduction in the numbers of eggs ovulated. Although not part of this study, observations show that the eggs which are produced, are capable of normal fertility.

It has been shown that ovulation rate is proportional to the duration of oestrus (Land, 1970; Hannahan and Quirke, 1975), which in turn can be influenced by exogenous oestrogens (Scaramuzzi *et al*., 1971a; Land *et al*., 1972). The administration of exogenous steroid oestrogens increases the length of the oestrus period. However, a single administration of exogenous oestrogen is most effective, as ewes show refractoriness to repeated doses (Scaramuzzi *et al*., 1972).

Morley et al., (1963) found that daily administration of stilboestrol to cycling ewes decreased ovulation rates, a response which parallels that of the coumestans. It may be that a single, appropriately-timed dose of coumestans will increase the duration of oestrus and increase ovulation rates, as does a single dose of steroid oestrogens.

Although Newsome and Kitts (1977) found that the ingestion of coumestans reduced the levels of oestradiol, oestrone and oestriol which bound to receptors, they did not measure total free endogenous oestrogen levels. Scaramuzzi (1975) and Fairclough et al., (1976) found that the depression of endogenous oestrogen levels, by the administration of anti-sera, prevented the occurrence of behavioural oestrus. The coumestans do not significantly increase the incidence of 'silent' heats. It is also unlikely that the coumestans are involved in the expression of behavioural oestrus and therefore mask lowered endogenous oestrogen levels, as immunization against phyto-oestrogens does not prevent oestrus (Cox and Wilson, 1976).

The demonstration in Experiment II, that the ingestion of oestrogenic lucerne late in the oestrous cycle depresses ovulation rates, whereas ingestion early in the cycle has no effect, is analogous to the effects of hemicastration before or after day 14 of the cycle. Unilateral ovariectomy before day 14 results in a compensatory increase in follicular growth in the remaining ovary, and consequently, ovulation rates are unchanged from those of intact ewes (Ramirez and Sawyer, 1974; Findlay and Cumming, 1977). There is an increase in FSH levels following hemicastration and the subsequent fall in endogenous oestrogen levels (Findlay and Cumming, 1977). A similar relationship between changes in FSH levels and follicular development could be involved in the

coumestan-induced depression of ovulation rates. The ingestion of oestrogenic lucerne may depress FSH levels and consequently, inhibit the latter stages of follicle maturation, so decreasing the number of Graafian follicles likely to ovulate. Smith et al., (unpublished data) recently found that the administration of Pregnant Mares Serum Gonadotrophin (PMSG) to ewes grazing oestrogenic lucerne, would overcome the coumestan-induced depression in ovulation rates.

A depression of FSH secretion by the coumestans could arise from either the direct action of the coumestans on the pituitary, in a manner similar to the negative-feedback action of the endogenous oestrogens, or from a coumestan-induced increase in endogenous oestrogen levels. Although Newsome and Kitts (1977) showed that the coumestans depressed the levels of receptor-bound endogenous oestrogens, they did not measure levels of 'free' endogenous oestrogens. The coumestans may effectively increase plasma oestrogen levels acting at the hypothalamo-pituitary level, by reducing the amount of endogenous oestrogens bound to reproductive tract oestrogen receptors over the peri-oestrus period. It has been demonstrated that the coumestans compete with endogenous oestrogens for oestrogen receptors (Shemesh et al., 1972).

Alternatively, the coumestans may lower plasma endogenous oestrogen levels and affect the pituitary response to Gn-RH. A strong correlation between the oestradiol : progesterone ratio and the pituitary response to Gn-RH has been reported (Chamley et al., 1974a; 1974b; Pelletier, 1976). Coumestans may alter gonadotrophin secretion patterns by lowering the oestradiol : progesterone ratio and consequently, the response to Gn-RH.

Findlay and Cumming (1977) suggested that FSH secretion is much

more sensitive to the negative-feedback action of oestrogen than is LH. Such variation in sensitivity has been demonstrated with the administration of exogenous oestrogens (Salamonsen et al., 1973; Baird and Scaramuzzi, 1976). If there is such a wide variation in response to coumestans, a fall in FSH levels of sufficient magnitude to influence ovulation rates may have occurred, while the changes in LH secretion were not large enough to be detected by the procedures used in this trial.

The ingestion of coumestans depresses ovulation rates but seldom prevents ovulation from occurring. How could a depression in FSH levels account for this? It has been shown that the presence of FSH leads to an increase in the number of FSH receptors; that FSH and oestradiol in concert increase LH receptor levels, and that LH increases LH receptor levels (Richards and Midgley, 1976). It is feasible that the depression of FSH (and the subsequent increase in LH:FSH ratio) after exposure to coumestans, will result in a depression in FSH receptor levels and an increase in LH receptor levels within developing follicles. Presumably, as FSH levels are limiting, the available FSH will be taken up preferentially by the follicle (or follicles) at the highest stage of development, which will possess the largest number of FSH receptors. This will in turn lead to an increase in FSH receptor levels and consequently, to greater FSH uptake. This 'cascading' effect will result in the largest follicle (or follicles) developing to the stage of potential ovulation, at the expense of other Graafian follicles.

The increased susceptibility of high fecundity breeds to the coumestans may be related to FSH secretion. Although there is no

evidence to show that ewes of high fecundity have high plasma FSH levels, it has been shown that such ewes are less sensitive to the negative-feedback actions of exogenous oestrogens (Land et al., 1972; 1976; Land, 1976). If these ewes are also less sensitive to endogenous oestrogens, they would presumably have higher FSH levels, a lower LH:FSH ratio, and as a consequence of these plasma gonadotrophin levels, would develop more follicles with the potential to ovulate. The more follicles with this potential to ovulate that are produced in normal ewes, the greater is the possible influence of the coumestans.

Direct evidence in support of this postulated action of the coumestans awaits further experimentation.

POSSIBLE FUTURE TRIALS

Further work needs to be done to determine the extent to which FSH is implicated in the depression of ovulation rates, in ewes grazing oestrogenic lucerne.

If a depression in FSH secretion does occur in ewes grazing oestrogenic lucerne, and FSH is responsible for follicular hypertrophy after hemicastration, then if hemicastrated ewes are subjected to coumestans, follicular hypertrophy will be impaired. Trials involving hemicastration and oestrogenic lucerne treatments at different times in the oestrous cycle could determine this. The administration of PMSG to hemicastrated, or intact ewes, at different times during the cycle may add further support to the claim that FSH is involved in determining ovulation rates.

Further assays of plasma gonadotrophin levels in ewes grazing oestrogenic lucerne, are necessary. These should involve intensive blood sampling regimes which would enable accurate quantitative

secretion rates to be calculated. Investigations into the mechanisms which influence gonadotrophin secretion, if plasma gonadotrophin levels are altered by the coumestans, could include the estimation of plasma endogenous oestrogen levels and the responses to exogenous Gn-RH of ewes grazing oestrogenic lucerne.

PRACTICAL APPLICATIONS OF THIS INVESTIGATION

In those areas of New Zealand which experience dry summers, lucerne often outproduces pasture in late summer and autumn. Lucerne may be the only available forage prior to mating, when it is important to maintain breeding ewes at high liveweights.

The results obtained in these trials suggest that oestrogenic lucerne may be utilized to achieve optimum pre-mating ewe liveweights, without any detrimental effects on reproductive performance if ewes are removed from the lucerne eight days prior to mating. In fact removal from lucerne as late as three days prior to the onset of oestrus may be all that is required to restore normal fecundity. In practical terms, it could be recommended to remove ewes from oestrogenic lucerne immediately prior to mating, as only a small percentage of ewes would exhibit oestrus within this three day period. Alternatively, it could be suggested to remove ewes a week prior to mating.

Further work involving much larger numbers of ewes, needs to be conducted to verify that removal from lucerne at mid-cycle restores normal ovulation rates, and hence lambing rates. Similarly, the effects of longer term grazing of oestrogenic lucerne prior to removal (such as for 1-2 months pre-mating), must also be investigated before these findings are strongly advocated.

BIBLIOGRAPHY

B I B L I O G R A P H Y

Adams, N.R. (1973). Relationship between uterine cysts and ovarian activity in Western Australian ewes. Aust. Vet. J. 49: 176.

Adams, N.R. (1976a). Cervical mucus changes in infertile ewes previously exposed to oestrogenic subterranean clover. Res. Vet. Sci. 21: 59-63.

Adams, N.R. (1976b). Altered ovarian function in ewes after prolonged exposure to plant oestrogens. Theriogenology. 6: 625.

Adams, N.R. (1977). Cervical mucus and reproductive efficiency in ewes after exposure to oestrogenic pastures. Aust. J. Agric. Res. 28: 481-489.

Adams, N.R. (1978). Presistently altered sensitivity to oestrogens in ewes after prolonged exposure to plant oestrogens. Abst. 6th Asia and Oceania Cong. Endocrinol pp.71-72.

Adler, J.H. and Weitzkin-Neiman, G. (1970). Demethylation of methyl-O-coumestrol by rumen micro-organisms in vitro. Refuah. Vet. 27: 51-55.

Alexander, G. and Rossiter, R.C. (1952). The effects of fertilizer treatments on the oestrogenic potency of Trifolium subterraneum. Aust. J. Agric. Res. 3: 24-28.

Allen, D.M. and Lamming, G.E. (1961). Nutrition and reproduction in the ewe. J. Agric. Sci. (Camb.) 56: 69-79.

Allen, E. and Doisy, E.A. (1923). An ovarian hormone: preliminary report on its localization, extraction and partial purification and action on test animals. J. Amer. Med. Assn. 81: 819-821.

Allison, A.J. (1968). The influence of live weight on ovulation rate in the ewe. Proc. N.Z. Soc. Anim. Prod. 28: 115-119.

Allison, A.J. (1972). Production of cervical mucus in ewes treated with exogenous progestagen and oestrogen. Aust. J. Agric. Res. 23: 473-481.

Anderson, J.N., Peck, E.J., and Clark, J.H. (1975). Estrogen-induced uterine responses and growth: Relationship to receptor estrogen binding by uterine nuclei. Endocrinol. 96: 160-167.

Armstrong, D.T., Grinwich, D.L., Moon, Y.S. and Zamecnik, J. (1974). Inhibition of ovulation in rabbits by intrafollicular injection of indomethacin and prostaglandin F. antisera. Life Sci. 14: 129-140.

Bahr, J., Kao, L. and Nalbandov, A.V. (1974). The role of the catecholamines and nerves in ovulation. Biol. Reprod. 10: 273-290.

Baird, D.T., Baker, T.G., McNatty, K.P. and Neal, P. (1975). Relationship between the secretion of the corpus luteum and the length of the follicular phase of the ovarian cycle. J. Reprod. Fert. 45: 611-619.

Baird, D.T. and Scarmuzzi, R.J. (1976). Changes in the secretion of ovarian steroids and pituitary LH in the peri-ovulatory period in the ewe; the effect of progesterone. J. Endocrinol. 70: 237-245.

Baird, D.T., Swanston, I. and Scaramuzzi, R.J. (1976). Pulsatile release of LH and secretion of ovarian steroids in sheep during the luteal phase of the oestrous cycle. Endocrinol. 98: 1490-1496.

Barcikowski, B., Carlson, J.C., Wilson, L. and McCracken, J.A. (1974). The effects of endogenous and exogenous oestradiol-17β on the release of prostaglandin $F_{2\alpha}$ from the ovine uterus. Endocrinol. 95: 1340-1349.

Barrell, G.K. (1976). Studies of neuroendocrine mechanisms influencing seasonal variations in semen production and plasma hormone levels in rams. Ph.D. Thesis. Massey University.

Barrett, J.F., George, J.M. and Lamond, D.R. (1965). Reproductive performance of Merino ewes grazing red clover (Trifolium pratense L) improved pasture or native pasture. Aust. J. Agric. Res. 16: 189-200.

Barrett, J.F., Moule, G.R., Braden, A.W.H. and Harris, A.N.A. (1961). Cystic glandular hyperplasia of the endometrium in ewes in New South Wales and Queensland. Aust. Vet. J. 37: 14.

Batterham, T.J., Hart, N.K., Lamberton, J.A. and Braden, A.W.H. (1965). Metabolism of oestrogenic isoflavones in sheep. Nature. 206: 509.

Batterham, T.J., Shutt, D.A., Hart, N.K., Braden, A.W.H. and Tweedale, H.J. (1971). Metabolism of intraruminally administered 4- ^{14}C-formononetin and 4- ^{14}C-biochanin A in sheep. Aust. J. Agric. Res. 22: 131-138.

Beck, A.B. and Knox, J.R. (1971). The acylated isoflavone glycosides from subterranean clover and red clover. Aust. J. Chem. 24: 1509-1518.

Beck, T.W. and Reeves, J.J. (1973). Serum LH in ewes treated with various dosages of 17β- oestradiol at three stages of the anoestrous season. J. Anim. Sci. 36: 566-570.

Bennet, D. (1966). The effect of stilboestrol diproprionate and leguminous pastures on the mammary secretion of ewes. Aust. Vet. J. 42: 364-368.

Bennett, D., Morley, F.W.H. and Axelsen, A. (1967a). Bioassay response of ewes to legume swards: 1. Uterine weight response; variability, calibration and prediction. Aust. J. Agric. Res. 18: 485-494.

Bennett, D., Morley, F.W.H. and Axelsen, A. (1967b). Bioassay responses of ewes to legume swards: 2. Uterine weight results from swards. Aust. J. Agric. Res. 18: 495-504.

Bennetts, H.W., Underwood, E.J. and Shier, F.L. (1946). A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Aust. Vet. J. 22: 2-11.

Bherer, J., Matton, P. and Dufour, J. (1977). Fate of the two largest follicles in the ewe after injection of gonadotrophins at two stages of the oestrous cycle. Proc. Soc. Exp. Biol. Med. 154: 412-414.

Bickoff, E.M., Booth, A.N., Lyman, R.L., Livingston, A.L., Thompson, C.R. and De Eds, F. (1957). Coumestrol, a new oestrogen isolated from forage crops. Science. 126 : 969-970.

Bickoff, E.M., Booth, A.N., Livingstone, A.L. and Hendrickson, A.P. (1960a). Observations of the effects of drying on oestrogenic activity of alfalfa samples of varying maturity. J. Anim. Sci. 19 : 745-753.

Bickoff, E.M., Booth, A.N., Livingstone, A.L., Hendrickson, A.P. and Lyman, R.L. (1959). Determination of estrogenic activity in fresh and dried forage. J. Anim. Sci. 18 : 1000-1009.

Bickoff, E.M., Booth, A.N., Lyman, R.L., Livingston, A.L., Thompson, C.R. and Kohler, G.O. (1958). Isolation of a new estrogen from Ladino clover. J. Agric. Food Chem. 6 : 536-539.

Bickoff, E.M., Livingstone, A.L., Booth, A.N., Thompson, C.R., Hollowell, E.A. and Beinhart, E.G. (1960b). Some variation in estrogenic activity in fresh and dried white clover clones and the Ladino variety. J. Anim. Sci. 19 : 1143-1149.

Bickoff, E.M., Livingstone, A.L., Hendrickson, A.P. and Booth, A.N. (1962). Relative potencies of several oestrogen-like compounds found in forages. J. Agric. Food Chem. 10 : 410-412.

Bickoff, E.M., Livingstone, A.L. and Guggolz, J. (1965). Isolation of phenolic compounds from Ladino clover. J. Agric. Food Chem. 13 : 151-155.

Bickoff, E.M., Livingston, A.L. and Witt, S.C. (1964). Isolation of coumestrol and other phenolics from alfalfa by countercurrent distribution. J. Pharm. Sci. 53 : 1496-1499.

Bindon, B.M., Chang, T.S. and Newton-Turner, H. (1971). Ovarian response to gonadotrophins by Merino ewes selected for fecundity. Aust. J. Agric. Res. 22 : 809-820.

Bjersing, L., Hay, M.F., Kann, G., Moor, R.M., Naftolin, F., Scaramuzzi, R.J., Short, R.V. and Younglai, E.V. (1972). Changes in gonadotrophins, ovarian steroids and follicular morphology in sheep at oestrus. J. Endocrinol. 52 : 465-479.

Bognadove, E.M. (1964). The role of the brain in the regulation of pituitary gonadotrophin secretion. Vit. and Horm. 22 : 206-260.

Bolt, D.J. and Hawk, H.W. (1971). C.L. regression induced by E_1 $E^{2\beta}$ and DES in ewes. J. Anim. Sci. 33 : 249.

Bolt, D.J., Kelly, H.E. and Hawk, H.W. (1971). Release of LH by oestradiol in cycling ewes. Biol. Reprod. 4 : 35-40.

Bradbury, R.B. and White, D.E. (1951). The chemistry of subterranean clover. 1. The isolation of formononetin and genistein. J. Chem. Soc. 1951 : 3447-3449.

Bradbury, R.B. and White, D.E. (1954). Estrogens and related substances in plants. Vit. and Horm. 12 : 207-233.

Braden, A.W.H., Hart, N.K. and Lamberton, J.A. (1967). The oestrogenic activity and metabolism of certain isoflavones in sheep. Aust. J. Agric. Res. 18 : 335-349.

Bradford, G.E., Quirke, J.F. and Hart, R. (1971). Natural and induced ovulation rate of Finnish Landrace and other breeds of sheep. Anim. Prod. 13 : 627-635.

Brand, A. and De Jong, W.H.R. (1973). Qualitative and quantitative micromorphological investigations of the tertiary follicle population during the oestrous cycle in sheep. J. Reprod. Fert. 33 : 431-439.

Brien, F.D., Baxter, R.W., Findlay, J.K. and Cumming, I.A. (1976). Effect of lupin grain supplementation on ovulation rate and plasma FSH concentration in maiden and mature Merino ewes. Proc. Aust. Soc. Anim. Prod. 11 : 237-240.

Butenandt, A. and Jacobi, H. (1933). Uber die Darstellung eines krystallisierten pflazlichen Tokokinins (Thelykinins) und seine Identifizierung mit dem α-Follikelhormon. Z. Physiol. Chem. 218 : 104-112.

Butler, G.W., Steemers, M.A. and Wong, E. (1967). The effect of notrogen, phosphorus and potassium supply on the isoflavone content of leaves of red clover (Trifolium pratense). N.Z. J. Agric. Res. 10 : 312-315.

Capony, F. and Rochefort, H. (1977). In vitro and In vivo interactions of (^{3}H) dimethylstilboestrol with the estrogen receptor. Mol. and Cell. Endocrinol. 8 : 47-64.

Carlson, J.C., Barcikowski, B., Cargill, V. and McCracken, J.A. (1974). The blockade of LH release by indomethacin. J. Clin. Endocrinol. Metab. 39 : 399-402.

Carson, R.S., Findlay, J.K. and Burger, H.G. (1978). Gonadotrophin receptors within the ovine ovarian follicle. Absts. 6th Asia and Oceania Cong. Endocrinol. 197-198.

Cayen, M.N. and Common, R.H. (1965). An investigation of urinary conversion products of coumestrol in the fowl. Biochem. Biophys. Acta. 100 : 567-573.

Chamley, W.A., Findlay, J.K., Cumming, I.A., Buckmaster, J.M. and Goding, J.R. (1974a). Effect of pregnancy on LH response to synthetic gonadotrophin-releasing hormone in the ewe. Endocrinol. 94 : 291-293.

Chamley, W.A., Findlay, J.K., Jonas, H., Cumming, I.A. and Goding, J.R. (1974b). Effect of pregnancy on the FSH response to synthetic gonadotrophin-releasing hormone in ewes. J. Reprod. Fert. 37 : 109-112.

Chamley, W.A., Cumming, I.A. and Goding, J.R. (1974c). Studies on oestrogen binding to uterine cytosol receptors prepared from normal and clover-infertile ewes. J. Reprod. Fert. 36 : 491.

Chang, M.C. (1966). Transport of eggs from the Fallopian tube to the uterus as a function of oestrogen. Nature 212: 1048-1049.

Chang, T.S. (1963). Genotype-pasture interaction in the fertility of Romney Marsh ewes. Proc. N.Z. Soc. Anim. Prod. 23 : 100-106.

Channing, C.P. (1970). Influences of the In vivo and In vitro hormonal environment upon luteinization in tissue culture. Recent Prog. Horm. Res. 26 : 589-622.

Cheng, E., Story, C.D., Payne, L.C., Yoder, L. and Burroughs, W.J. (1953). Detection of oestrogenic substances in alfalfa and clover hays fed to fattening lambs. J. Anim. Sci. 12 : 507-514.

Cheng, E., Yoder, L., Story, C.D. and Burroughs, W.J. (1954). Oestrogenic activity of some isoflavone derivaties. Science 120 : 575-576.

Cidlowski, J.A. and Muldoon, T.G. (1976). Dissimilar effects of anti-estrogens upon estrogen receptors in responsive tissues of male and female rats. Biol. Reprod. 15 : 381-389.

Cidlowski, J.A. and Muldoon, T.G. (1978). The dynamics of intracellular estrogen receptor regulations as influenced by 17β-estradiol. Biol. Reprod. 18 : 234-246.

Clark, J.H., Paszko, Z and Peck, E.J. (1977). Nuclear binding and retention of the receptor-oestrogen complex : Relation of the agonistic and antagonistic properties of estriol. Endocrinol. 100 : 91-96.

Clark, N.T. (1965). A comparison of the reproductive performance of ewes grazing Dwalganup or Bacchus Marsh subterranean clover or a mixture of lucerne and Burr medic before and during mating. Aust. J. Exptal. Agric. 5 : 106-109.

Close, R.C. (1967). Disease of lucerne in New Zealand. From 'The Lucerne Crop'. ed. R.H.M. Langer. Published: A.H.& A.W. Reed. Wgtn. N.Z.

Coleman, E.A., Stob, M. and Keim, W.F. (1965). Uterotrophic activity in Lotus corniculatus L. Crop Sci. 5: 276-277.

Coop, I.E. (1966a). Effect of flushing on reproductive performance of ewes. J. Agric. Sci. 67: 305-323.

Coop, I.E. (1966b). The response of ewes to flushing. World Rev. Anim. Prod. 2 (4): 69-74.

Coop, I.E. (1977). Depression of lambing percentage from mating on lucerne. Proc. N.Z. Soc. Anim. Prod. 37: 149-151.

Coop, I.E. and Clark, V.R. (1960). The reproductive performance of ewes mated on lucerne. N.Z. J. Agric. Res. 3: 922-933.

Costello, C.H. and Lynn, E.V. (1950). An oestrogenic compound from liquorice root extract. J. Amer. Pharm. 39: 177-181.

Cox, R.I. and Braden, A.W.H. (1974). The metabolism and physiological effects of phyto-oestrogens in livestock. Proc. Aust. Soc. Anim. Prod. 10: 122-129.

Cox, R.I., Mattner, P.E. and Thorburn, G.D. (1971). Changes in ovarian secretion of oestradiol-17β around oestrus in the sheep. J. Endocrinol. 49: 345-346.

Cox, R.I., Thorburn G.D., Currie, W.B. and Restall, B.J. (1974). Interrelationships between oestrogens and prostaglandin F concentrations in the utero-ovarian venous plasma of the ewe. J. Reprod. Fert. 36: 448-449.

Cox, R.I. and Wilson, P.A. (1976). Effects of immunizing sheep to steroid oestrogens and phyto-oestrogens. J. Reprod. Fert. 46: 524.

Cumming, I.A. (1977). Relationships in the sheep of ovulation rate with liveweight, breed, season and nutrition. Aust. J. Exptal. Agric. and Anim. Husb. 17: 234-241.

Cumming, I.A., Buckmaster, J.M., Blockey, M.A., Goding, J.R., Winfield C.G. and Baxter, R.W. (1973). Constancy of interval between LH release and ovulation in the ewe. Biol. Reprod. 9 : 24-29.

Cumming, I.A. and McDonald, M.F. (1967). The production of ova by New Zealand Romney ewes following hormonal stimulation. N.Z. J. Agric. Res. 10 : 226-236.

Curnow, D.H., Robinson, T.J. and Underwood, E.J. (1948). Oestrogenic action of extracts of subterranean clover (T. subterranean L. var. Dwalganup). Aust. J. Exptal Biol. Med. Sci. 26 : 171-180.

Curnow, D.H. and Rossiter, R.C. (1955). The occurrence of genistein in subterranean clover (T. subterranean L) and other Trifolium species. Aust. J. Exptal Biol. Med. Sci. 33 : 243-248.

Dattagupta, P.C., Khastgir, H.N. and Sengupta, P. (1960). Investigations on the structure of psoralidin. Chem. and Ind. 1960a : 48-49.

Davies, H.L. and Dudzinski, M.L. (1965). Further studies on oestrogenic activity in strains of subterranean clover (Trifolium subterranean L) in south-western Australia. Aust. J. Agric. Res. 16 : 937-950.

Davies, H.L., Rossiter, R.C. and Maller, R. (1970). The effects of different cultivars of subterranean clover (T. subterranean L) on sheep reproduction in the south-west of Western Australia. Aust. J. Agric. Res. 21 : 359-369.

Diekman, M.A. and Malvern, P.V. (1973). Effect of ovariectomy and oestradiol on LH patterns in ewes. J. Anim. Sci. 37 : 562-567.

Dorhn, M. Faure, W., Poll, H. and Blotevogel, W. (1926). - cited by K. Kallela, Thesis, Helsinki, 1964. Med. Klin. (Munich) 22 : 1417.

Ducker, M.J. and Boyd, J.S. (1977). The effect of body size and body condition on the ovulation rate of ewes. Anim. Prod. 24 : 377-385.

Dufour, J. (1976). Caracteristiques des follicules ovariens lors de l'ovulation simple ou double chez le brebis et developpment de leurs corps jaunes. Informations Veterinaires 18 : 16-17.

Dufour, J., Ginther, O.J. and Casida, L.E. (1971a). Compensatory hypertrophy after unilateral ovariectomy and the destruction of follicles in the anoestrous ewe. Proc. Soc. Exptal Biol. Med. 138 : 1068-1072.

Dufour, J., Ginther, O.J. and Casida, L.E. (1971b). Corpus luteum action on ovarian follicular development after destruction of macroscopically visible follicles in ewes. Proc. Soc. Exptal. Biol. Med. 138 : 475-478.

Dufour, J. and Matton, P. (1977). Identification of ovarian follicles at estrus and development of their ensuing corpora lutea in single and multiple ovulating ewes on two feeding regimes. Can. J. Anim. Sci. 57 : 647-652.

East, J., Underwood, E.J. and Bennetts, H.W. (1949). Oestrogenic effects of subterranean clover (T subterranean L var. Dwalganup) : Protective action of androgens in the castrate male. Aust. J. Exptal. Biol. Med. Sci. 27 : 105-113.

Eastwood, K.C. and McDonald, M.F. (1975). Reproductive differences in two-year-old Romney and Border Leicester cross Romney ewes after gonadotrophin treatment. N.Z.J. Agric. Res. 18: 109-117.

Echternkamp, S.E. and Laster, D.B. (1976). Plasma LH concentrations for pre-puberal, post-puberal, anoestrous and cycling ewes of varying fecundity. J. Anim. Sci. 42 : 444-447.

Edey, T.N. (1968). Bodyweight and ovulation rate in sheep. Proc. Aust. Soc. Anim. Prod. 7 : 188-191.

Edwards, R.G. (1974). Follicular fluid. J. Reprod. Fert. 37 : 189-219.

Espey, L.L. (1974). Ovarian proteolytic enzymes and ovulation. Biol. Reprod. 10 : 216-235.

Espey, L.L. and Coons, P.J. (1976). Factors which influence ovulatory degradation of rabbit ovarian follicles. Biol. Reprod. 14 : 233-245.

Fairclough, R.J., Smith, J.F. and Peterson, A.J. (1976). Passive immunization against oestradiol-17β and its effect on luteolysis, oestrus and ovulation in the ewe. J. Reprod. Fert. 48 : 169-171.

Farkas, G.L. and Kiraly, Z. (1962). Role of phenolic compounds in the physiology of plant diseases and disease resistance. Phytopath. Z. 44 : 105-109.

Fellner, O.O. (1926). - cited by K. Kallela, Thesis, Helsinki, 1964. Med. Klin. (Munich) 22 : 1886-1888.

Ferguson, E.R. and Katzenellenbogen, B.S. (1977). A comparative study of anti- estrogen action : Temporal patterns of antagonism of estrogen-stimulated uterine growth and effects of estrogen receptor levels. Endocrinol. 100 : 1242-1251.

Findlay, J.K. and Cumming, I.A. (1976a). FSH in the ewe : Effects of season, liveweight and plane of nutrition on plasma FSH and ovulation rate. Biol. Reprod. 15 : 335-342.

Findlay, J.K. and Cumming, I.A. (1976b). Increases in ovulation rate in sheep following administration of an LH-RH analogue. Biol. Reprod. 15 : 115-117.

Findlay, J.K. and Cumming, I.A. (1977). The effect of unilateral ovariectomy on plasma gonadotrophin levels, estrus and ovulation rate in sheep. Biol. Reprod. 17 : 178-183.

Findlay, J.K., Cumming, I.A., Chamley, W.A., Buckmaster, J.M., Goding, J.R. and Hearnshaw, H. (1973). The release of LH by oestradiol-17β and a synthetic Gn-RH in ewes affected with Clover Disease. J. Reprod. Fert. 32 : 341.

Firth, J.H.., Salamon, S. and Maxwell, W.M.C. (1977). Observations on oestrus without ovulation in sheep. Proc. Aust. Soc. Biol. Reprod. 9: 58.

Fletcher, I.C. (1971). Effects of nutrition, liveweight and season on the incidence of twin ovulation in South Australian strong wool Merino ewes. Aust. J. Agric. Res. 22: 321-330.

Fletcher, I.C. and Lindsay, D.R. (1971a). Effect of oestrogen on oestrus behaviour and its variation with season in the ewe. J. Endocrinol 50: 685-696.

Fletcher, I.C. and Lindsay, D.R. (1971b). Effect of rams on the duration of oestrus behaviour in ewes. J. Reprod. Fert. 25: 253-259.

Ford, S.P., Weems, C.W. , Pitts, R.E., Pexton, J.E., Butcher, R.L. and Inskeep, E.K. (1975). Effects of oestradiol-17β and progesterone on prostaglandinF in sheep uteri and uterine venous plasma. J. Anim. Sci. 41: 1407-1413.

Foster, J.P. and Crighton, D.B. (1974). Luteinizing Hormone (LH) release after single injections of synthetic LH-releasing hormone (LH-RH) in the ewe at three different reproductive stages and comparisons with natural LH release at oestrus. Theriogenology. 2: 87-100.

Francis, C.M. and Millinton, A.J. (1964). Varietal variation in the isoflavone content of subterranean clover: Its estimation by a microtechnique. Aust. J. Agric. Res. 16: 557-564.

Francis, C.M. and Millington, A.J. (1965a). Wether bioassay of annual pasture legumes: IV. The oestrogenic activity of annual medic pastures. Aust. J. Agric. Res. 16: 927-935.

Francis, C.M. and Millington, A.J. (1965b). Isoflavone mutations in subterranean clover: I. Their production, characteristics and inheritance. Aust. J. Agric. Res. 16: 565-573.

Francis, C.M. and Millington, A.J. (1965c). Wether bioassay of annual pasture legumes: III. The oestrogenic potency of dry subterranean clover pastures, and of leaf blade and petiole in the green state. Aust. J. Agric. Res. 16: 23-30.

Francis, C.M., Millington, A.J. and Bailey, E.T. (1967). The distribution of oestrogenic isoflavones in the genus Trifolium. Aust. J. Agric. Res. 18: 47-54.

Gibbons, R.A. and Mattner, P. (1966). Some aspects of the chemistry of cervical mucus. Int. J. Fert. 11: 366-372.

Goding, J.R., Blockey, M.A., Brown, J.M., Catt, K.J. and Cumming, I.A. (1970). The role of oestrogen in the control of the oestrous cycle in the ewe. J. Reprod. Fert. 21: 368-369.

Goding, J.R., Catt, K.J., Brown, J.M., Kaltenbach, C.C., Cumming, I.A. and Mole, B.J. (1969). R.I.A. for ovine LH: Secretion of LH hormone during oestrus and following oestrogen administration in the sheep. Endocrinol. 85: 133-142.

Gorski, J. and Gannon, F. (1976). Current models of steroid hormone action; a critique. Ann. Rev. Physiol. 38: 425-450.

Grisebach, H. and Barz, W. (1963). The biogenesis of coumestrol in lucerne. Zietschrift Fur Naturforschung. 18B 466-470.

Guggolz, J., Livingstone, A.L. and Bickoff, E.M. (1961). Detection of diadzein, formononetin, genistein and biochanin A in forages. J. Agric. Food Chem. 9: 330-332.

Hamada, Y., Bronson, R.A., Wright, K.H. and Wallach, E.E. (1977). Ovulation in the perfused rabbit ovary; the influence of prostaglandins and prostaglandin inhibitors. Biol. Reprod. 17: 58-63.

Hammond, J. (1945). Induced ovulation and heat in anoestrus sheep. J. Endocrinol. 4: 169-180.

Hanrahan, J.P. and Quirke, J.F. (1975). Repeatibility of the duration of oestrus and breed differences in the relationships between duration of oestrus and ovulation rate in sheep. J. Reprod. Fert. 45: 29-36.

Hanson, C.H., Loper, G.M., Kohler, G.O., Bickoff, E.M. and Taylor, K.N. (1965) Variation in coumestrol content of alfalfa as related to location, variety, cutting, year, stage of growth and disease. U.S.D.A. Tech . Bull. 1333.

Harned, M.A. and Casida, L.E. (1971). Local response to progesterone in the ewe ovary. J. Anim. Sci. 33 : 1158.

Hawk, H.W. (1974). Oestrogenic effects on uterine motility in ewes. J. Anim. Sci. 39 : 209-210.

Hawk, H.W. (1975). Enhancement by exogenous oestradiol of uterine motility in oestrous ewes. J. Anim. Sci. 41 : 572-577.

Hawk, H.W. and Bolt, D.J. (1970). Luteolytic effect of oestradiol-17β when administered after mid-cycle in the ewe. Biol. Reprod. 2 : 275-278.

Hawk, H.W. and Cooper, B.S. (1975). Improvement of sperm transport by the administration of oestradiol to oestrous ewes. J. Anim. Sci. 41 : 1400-1406.

Hawk, H.W. and Cooper, B.S. (1976). Ovum fertilization and embryo survival in ewes treated with oestradiol immediately prior to mating. J. Anim. Sci. 42 : 677-680.

Hay, M.F. and Moor, R.M. (1975a). Distribution of Δ5-3β-hydroxysteroid dehydrogenase activity in the Graafian follicle of the sheep. J. Reprod. Fert. 43 : 313-322.

Hay, M.F. and Moor, R.M. (1975b). Functional and structural relationships in the Graafian follicle population of the sheep ovary. J. Reprod. Fert. 45 : 583-593.

Heape, W. (1899). Abortions, barrenness and fertility in sheep. J. Roy. Agric. Soc. 60 : 217-248.

Hearnshaw, H., Brown, J.M., Cumming, I.A., Goding, J.R. and Nairn, M. (1972). Endocrinological and histopathological aspects of the infertility in the ewe caused by oestrogenic clover. J. Reprod. Fert. 28 : 160-161.

Hearnshaw, H., Cumming, I.A. and Goding, J.R. (1977). Effect of ingestion of oestrogenic clover on Luteinizing Hormone release in the ovariectomized ewe. Aust. J. Biol. Sci. 30 : 217-223.

Hohlweg, W. (1934). Veranderungen des hypohysen-vorderlappens und des ovariums nach Behandlingen mit grossen-dosen von follikelhormon. Klin. Wchnshr 13 : 92-95.

Holst, P.J. and Braden, A.W.H. (1972). Ovum transport in the ewe. Aust. J. Biol. Sci. 25 : 167-173.

Hooley, R.D., Baxter, R.W., Chamley, W.A., Cumming, I.A., Jonas, H.A. and Findlay, J.K. (1974). FSH and LH response to gonadotrophin-releasing hormone during the ovine oestrous cycle and following progesterone administration. Endocrinol. 95 : 937-942.

Howland, B.E., Akbar, A.M. and Stormshak, F. (1971). Serum LH levels and luteal weight in ewes following a single injection of estradiol. Biol. Reprod. 5 : 25-29.

Howland, B.E. and Palmer, W.M. (1973). Estrogen-induced changes in serum luteinizing hormone levels in ovariectomized ewes. Can. J. Anim. Sci. 52 : 413-414.

Hseuh, A.J.W., Peck, E.J., and Clark, J.H. (1976). Control of uterine estrogen receptor levels by progesterone. Endocrinol 98 : 438-444.

Hulet, C.V., Price, D.A. and Foote, W.C. (1968). Effects of variation in light, month of year and nutrient intake on reproductive phenomena in ewes during the breeding season. J. Anim. Sci. 27 : 684-690

Hutchinson, J.S.M. and Robertson, H.A. (1966). The growth of the follicle and corpus luteum in the ovary of the sheep. Res. Vet. Sci., 7 : 17-24.

Ingham, J.L. (1972). Phytoalexins and other natural products as factors in plant disease resistance. The Botanical Rev. 38 : 343-424.

Jagusch, K.T., Smith, J.F. and Kelly, R.W. (1977). Effects of feeding lucerne during mating on the fertility of ewes. Proc. N.Z. Nutrition Soc. 1977: 26-28.

Jensen, E.V. and De Sombre, E.R. (1973). Estrogen-receptor interaction Science 182: 126-132.

Jonas, H.A., Salamonsen, L.A., Burger, H.A., Chamley, W.A., Cumming, I.A., Findlay, J.K. and Goding, J.R. (1973). Release of FSH after administration of Gn-RH or oestradiol to the anoestrous ewe. Endocrinol. 92: 862-865.

Jones, E.E. and Nalbandov, A.V. (1972). Effects of intrafollicular injection of gonadotrophins on ovulation or luteinization of ovarian follicles. Biol. Reprod. 7: 87-93.

Jones, H.E.H. and Pope, G.S. (1961). A method for the isolation of miroestrol from Peuraria mirifica. J. Endocrinol 22: 303-312.

Kallela, K. (1964). The incidence of plant oestrogens in Finnish pasture and fodder plants with special reference to their possible effects in cases of sterility in ruminants. Thesis. College of Veterinary Medicine, Helsinki, Finland.

Kaltenbach, C.C. and Davies, H.L. (1970). Fertilization, sperm transport and early embryonic loss in ewes grazed on cultivars of subterranean clover. (T. Subterranean) Aust. J. Agric. Res. 21: 107-115.

Katzenellenbogen, B.S., Ferguson, E.R. and Lan, N.C. (1977). Fundamental differences in the action of estrogens and anti-estrogens on the uterus: Comparison between compounds with similar duration of action. Endocrinol. 100: 1252-1259.

Kelly, R.W. (1972). The oestrogenic activity of coumestans in ovariectomized ewes. J. Reprod. Fert. 28: 159.

Kelly, R.W. (1976). Modified extraction technique for determination of amounts of coumestans in lucerne - pers comm. cited by McCutcheon, 1976. Invermay Agric. Res. Centre, Mosgiel, N.Z.

Kelly, R.W., Adams, N.R. and Lindsay, D.R. (1976a). Effect of coumestans on reproduction in the ewe. Aust. J. Agric. Res. 27 : 253-259.

Kelly, R.W., Allison, A.J. and Shirley, D.K. (1976b). Interactions between phyto-oestrogens and steroids in the cervical mucus and uterine weight responses in ewes. Aust. J. Agric. Res. 27 : 101-107

Killeen, I.D. (1967). The effects of bodyweight and level of nutrition before, during and after joining on ewe fecundity. Aust. J. Exptal Agric. Anim. Husb. 7 : 126-136.

Kohler, G.O. and Bickoff, E.M. (1961). Coumestrol and other biologically active compounds in forages. Feedstuffs 33 : 46.

Koligian, K.B. and Stormshak, F. (1977a). Progesterone inhibition of estrogen receptors replenishment in ovine endometrium. Biol. Reprod. 17 : 412-416.

Koligian, K.B. and Stormshak, F. (1977b). Nuclear and cytoplasmic estrogen receptors in ovine endometrium during the estrous cycle. Endocrinol. 101 : 524-533.

Krarup, T., Pederson, T. and Faber, M. (1969). Regulation of oocyte growth in the mouse ovary. Nature, 224 : 187-188.

Kuehl, F.A., Humes, J.L., Tarnoff, J., Cirillo, V.J. and Ham, E.A. (1970). Prostaglandin receptor sites; evidence for an essential role in the action of LH. Science. 169 : 883-886.

Lamming, G.E. (1978). Personal communication. Physiology Dept., School of Agric. Nottingham University.

Lan, N.C. and Katzenellenbogen, B.S. (1976). Temporal relationships between hormone receptor binding and biological responses in the uterus : Studies with short and long acting derivatives of estriol. Endocrinol. 98 : 220-227.

Land, R.B. (1970). A relationship between the duration of oestrus, ovulation rate and litter size of sheep. J. Reprod. Fert. 23 : 49-53.

Land, R.B. (1973). Ovulation rate in Finn-Dorset sheep following unilateral ovariectomy or chlorpromazine treatment, at different stages of the oestrous cycle. J. Reprod. Fert. 33 : 99-105.

Land, R.B. (1976). The sensitivity of the ovulation rate of Finnish Landrace and Scottish Blackface ewes to exogenous oestrogens. J. Reprod. Fert. 48 : 217-218.

Land, R.B., Pelletier, J., Thimonier, J. and Mauleon, P. (1973). A quantitative study of genetic differences in the incidence of oestrus, ovulation and plasma LH concentration in sheep. J. Endocrinol 58 : 305-317.

Land, R.B., Thompson, R. and Baird, D.T. (1972). The duration of oestrus in ovariectomized Finnish Landrace and Scottish Blackface ewes following progesterone and oestrogen treatment. J. Reprod. Fert. 30 : 39-44.

Land, R.B., Wheeler, A.G. and Carr, W.R. (1976). Seasonal variation in the oestrogen-induced LH discharge of ovariectomized Finnish Landrace and Scottish Blackface ewes. Ann. Biol. Anim. Bioch. Biophys. 16 : 521-528.

Lewis, P.E. and Warren, J.E. (1975). Indomethacin inhibits luteolysis in the ewe. J. Anim. Sci. 41 : 365-366.

Libertum, D., Orias, R. and McCann, S.M. (1974). Biphasic effect of oestrogen on the sensitivity of the pituitary to LH-RH Endocrinol. 94 : 1094-1100.

Lightfoot, R.J., Croker, K.P. and Neil, H.G. (1967). Failure of sperm transport in relation to ewe infertility following prolonged grazing on oestrogenic pastures. Aust. J. Agric. Res. 18 : 755-765.

Lightfoot, R.J., Marshall, T. and Croker, K.P. (1976). Effects of rate and duration of lupin grain supplementation on ovulation and fertility of Merino ewes. Proc. Aust. Soc. Anim. Prod. 11 : 5.p.

Lightfoot, R.J., Smith, J.F., Cumming, I.A., Marshall, T., Wroth, R.H. and Hearnshaw, H. (1974). Infertility in ewes caused by prolonged grazing of oestrogenic pastures : Oestrus, fertilization and cervical mucus. Aust. J. Biol. Sci. 27 : 409-414.

Lightfoot, R.J. and Wroth, R.H. (1974). The mechanism of temporary infertility in ewes grazing oestrogenic subterranean clover prior to and during joining. Proc. Aust. Soc. Anim. Prod. 10 : 130-134.

Lindner, H.R. (1967). Study of the fate of phyto-oestrogens in sheep by determination of isoflavones and coumestrol in the plasma and adipose tissue. Aust. J. Agric. Res. 18 : 305-333.

Lindsay, D.R., Cognie, Y., Pelletier, J and Signoret, J.P. (1975). Influence of the presence of rams on the timing of ovulation and discharge of LH in ewes. Physiol. and Behav. 15 : 423-426.

Lindsay, D.R. and Francis, C.M. (1968). Cervical mucus measurement in ovariectomized ewes as a bioassay of synthetic and phyto-oestrogens. Aust. J. Agric. Res. 19 : 1069-1076.

Lipner, H. and Greep, R.O. (1971). Inhibition of steroid synthesis at various sites in the biosynthetic pathway in relation to induced ovulation. Endocrinol. 88 : 602-617.

Livingstone, A.L., Bickoff, E.M., Guggolz, J. and Thompson, C.R. (1961). Quantitative determination of coumestrol in fresh and dried lucerne. J. Agric. Food Chem. 9 : 135-137.

Loewe, S., Lange, F. and Spohr, E. (1927) - cited by K. Kallela, Thesis, Helsinki, 1964. Biochem. Z. 180: 1.

Loper G.M. (1968). Effect of aphid infestation on the coumestrol content of alfalfa varieties differing in aphid resistance. Crop Sci. 8: 104-106.

Loper, G.M. and Hanson, C.H. (1964). Influence of controlled environmental factors and two foliar pathogens on coumestrol in alfalfa. Crop Sci. 4: 480-482.

Loper, G.M., Hanson, C.H. and Graham, J.H. (1967). Coumestrol content of alfalfa as effected by selection for resistance to foliar diseases. Crop Sci. 7: 189-192.

Luine, V.N. and McEwen, B.S. (1977). Effects of an estrogen antagonist on enzyme activities and (^{3}H) estradiol nuclear binding in the uterus, pituitary and brain. Endocrinol. 100: 903-910.

Lunenfield, B., Kraiem, Z. and Eshkol, A. (1975). The function of the growing follicle. J. Reprod. Fert. 45: 567-574.

Lyman, R.L., Bickoff, E.M., Booth, A.N. and Livingstone, A.L. (1959). Detection of coumestrol in Leguminous plants. Arch. Biochem. Biophys. 80: 61-67.

McCutcheon, S.N. (1976). A study of the reproductive performance of Border Leicester X Romney ewes mated on oestrogenic lucerne. B. Agric. Sci (Hons.) Dissertation, Massey University.

McDonald, M.F. and Chang, T.S. (1966). Variation in ovarian activity of Romney Marsh ewes. Proc. N.Z. Soc. Anim. Prod. 26: 98-106.

McInnes, P. and Smith, M.D. (1966). The effects of nutrition before mating on the performance of Merino ewes. Aust. J. Exptal Agric. and Anim. Husb. 6: 455-459.

McLean, J.W. (1967). Lucerne and phyto-oestrogens. From 'The Lucerne Crop' Ed. R.M. Langer. Published A.H. & A.W. Reed. Wgtn. N.Z.

Mallampati, R.S. and Casida, L.E. (1970). Ovarian compensatory hypertrophy following unilateral ovariectomy during the breeding season in the ewe. Biol. Reprod. 3: 43-46.

Marsh, J.M. (1971). The effects of prostaglandins on the adenyl cyclase of the bovine corpus luteum. Ann. New York Acad. Sci. 180: 416-425.

Mattner, P.R. (1966). Formation and retention of the spermatazoan reservoir in the cervix of the ruminant. Nature 212: 1479-1480.

Matton, P., Bherer, J. and Dufour, J.J. (1977). Morphology and responsiveness of the two largest ovarian follicles in anoestrous ewes. Can. J. Anim. Sci. 57: 459-464.

Micheli, R.A., Booth, A.N., Livingstone, A.L. and Bickoff, E.M. (1962). Coumestrol, plant phenolics and synthetic oestrogens; a correlation of structure and activity. J. Med. Pharm. Chem. 5: 321-325.

Miller, B.G., Murphy, L. and Stone, G.M. (1977b). Hormone receptor levels and hormone, RNA and protein metabolism in the genital tract during the oestrous cycle in the ewe. J. Endocrinol. 73: 91-98.

Miller, W.L., Knight, M.M., Grimek, H.J. and Gorski, J. (1977a). Estrogen regulation of Follicle Stimulating Hormone in cell cultures of sheep pituitaries. Endocrinol. 100: 1306-1316.

Millington, A.J., Francis, C.M. and McKeown, N.R. (1964a). Wether bioassay of annual pasture legumes. I. Oestrogenic activity in Medicago tribuloides desr. var. Cyprus relative to four strains of (Trifolium subterranean L.) Aust. J. Agric. Res. 15: 520-526.

Millington, A.J., Francis, C.M. and McKeown, N.R. (1964b). Wether bioassay of annual pasture legumes. II. The oestrogenic activity of nine strains of (Trifolium subterranean L.) Aust. J. Agric. Res. 15: 527-536.

Moor, R.M. (1973). Oestrogen production by individual follicles explanted from ovaries of sheep. J. Reprod. Fert. 32: 545-548.

Moor, R.M. (1974). The ovarian follicle of the sheep; inhibition of oestrogen secretion of luteinizing hormone. J. Endocrinol. 61: 455-463.

Moor, R.M. (1977). Sites of steroid production in ovine Graafian follicles in culture. J. Endocrinol. 73: 143-150.

Moor, R.M., Hay, M.F. and Caldwell, B.V. (1971). The sheep follicle; relation between the sites of steroid dehydrogenase activity, gonadotrophic stimulation and steroid production. J. Reprod. Fert. 27: 484-485.

Moore, N.W., Barret, S., Brown, J.B., Schindler, I., Smith, M.A. and Smyth, B. (1969). Oestrogen and progesterone content of ovarian vein blood of the ewe during the oestrous cycle. J. Endocrinol. 44: 55-62.

Moore, N.W. and Robinson, T.J. (1957). The behavioural and vaginal response of the spayed ewe to oestrogen injected at various times relative to the injection of progesterone. J. E ocrinol. 15: 360-365.

Morley, F.H.W., Axelsen, A. and Bennett, D. (1963). Effects of oestrogen on fertility in ewes. Nature. 199: 403-404.

Morley, F.H.W., Axelsen, A. and Bennett, D. (1964). Effects of grazing red clover (Trifolium pratense) during the joining season on ewe fertility. Proc. Aust. Soc. Anim. Prod. 5: 58-61.

Morley, F.H.W., Axelsen, A. and Bennett, D. (1966). Recovery of fertility after grazing on oestrogenic red clover. Aust. Vet. J. 42: 204-206.

Morley, F.H.W., Bennett, D., Braden, A.W.H., Turnbull, K.E. and Axelsen, A. (1968). Comparison of mice, guinea-pigs and sheep as test animals for bioassay of oestrogenic pasture legumes. Proc. N.Z. Soc. Anim. Prod. 28: 11-21.

Morris, I.D. (1976). Changes in brain, pituitary and uterine cytoplasmic oestrogen receptors induced by oestradiol-17β in the ovariectomized rats. J. Endocrinol. 71: 343-349.

Moule, G.R., Braden, A.W.H. and Lamond, D.R. (1963). The significance of oestrogens in pasture plants in relation to animal production. Anim. Brdg. Absts. 31: 139-157.

Murphy, L., Stone, G.M., Miller, B.G., and Moore, N.W. (1977). Oestradiol and progesterone: Soluble receptor levels and metabolism in the uterus of ovariectomized ewes. Aust. J. Biol. Sci. 30: 225-228.

Newsome, F.E. and Kitts, W.D. (1977). Effects of alfalfa consumption on oestrogen levels in ewes. Can. J. Anim. Sci. 57: 531-555.

Nilsson, A. (1961). On the In vitro metabolism of the plant oestrogen biochanin A in rumen fluid. Ark. Kemi 17: 305-310.

Nilsson, A. (1963). O-demethylation of biochanin A and some other isoflavones and methylated oestrogens by microsomal liver enzymes. Ark. Kemi. 21: 97-121.

Nilsson, A., Hill, J.A. and Davies, H.L. (1967). An In vitro study of formononetin and biochanin A metabolism in rumen fluid from sheep. Biochim. Biophys. Acta. 148: 92-98.

Niswender, G.D., Reichert, L.E., Midgley, A.R. and Nalbandov, A.V. (1969). Radioimmunoassay for bovine and ovine luteinising hormone. Endocrinol. 84: 1166-1173.

Obst, J.M. and Seamark, R.F. (1970). Plasma progesterone concentrations during the reproductive cycle of ewes grazing Yarloop clover. J. Reprod. Fert. 21: 545-547.

O'Malley, B.W. and Schrader, W.T. (1976). The receptors of steroid hormones. Sci. Amer. 234 (2): 32-43.

O'Shea, J.D. (1971). Smooth-muscle-like cells in the theca externa of ovarian follicles in the sheep. J. Reprod. Fert. 24: 283-285.

O'Shea, J.D. and Phillips, R.E. (1974). Contractility of ovarian follicles from sheep In vitro. J. Reprod. Fert. 36: 457.

Owman, C., Sjoberg, N.O., Svensson, K.G. and Walles, B. (1975). Autonomic nerves mediating contractility in the human Graafian follicle. J. Reprod. Fert. 45: 533-556.

Pant, H.C. (1977). Effect of oestradiol infusion on plasma gonadotrophins and ovarian activity in progesterone-primed and unprimed anoestrous ewes. J. Endocrinol. 75: 227-233.

Pelletier, J. (1976). Influence of LH-RH on LH and FSH release in domestic annimals. Ann. Biol. Anim. Biochm. Biophys. 16: 213-234.

Pelletier, J. and Signoret, J.P. (1970). pp. 215-223. from 'L'inhibition de l'ovulation' Ed. Masson et Cie.

Peters, H., Byskov, A.G., Lintern-Moore, S. and Faber, M. (1973). Follicle growth initiation in the immature mouse ovary; extra-ovarian or intra-ovarian ovarian control. J. Reprod. Fert. 35: 619-620.

Peters, H., Byskov, A.G., Himelstein-Braw, R. and Faber, M. (1975). Follicular growth; the basic event in the mouse and human ovary. J. Reprod. Fert. 45: 559-566.

Phelps, C.P., Coy, D.H., Schally, A.V. and Sawyer, C.H. (1977). Blockade of LH release and ovulation in the rabbit with inhibitory analogues of LH-RH. Endocrinol. 100 : 1526-1532.

Phi, L.T., Moon, Y.S. and Armstrong, D.T. (1977). Effects of systemic and intrafollicular injections of LH, prostaglandins and indomethacin on the luteinization of rabbit Graafian follicles. Prostaglandins 13 : 543-552.

Pieterse, P.J.S. and Andrews, F.N. (1956). The oestrogenic activity of alfalfa and other feedstuffs. J. Anim. Sci. 15 : 25-36.

Piper, E.L. and Foote, W.C. (1968). Ovulation and corpus luteum maintenance in ewes treated with 17β-oestradiol. J. Reprod. Fert. 16 : 253-259.

Pope, G.S. (1954). The importance of pasture plant oestrogens in the reproduction and lactation of grazing animals. Dairy Sci. Absts. 16 : 333-356.

Pope, G.S. and Wright, H.G. (1954). Oestrogenic isoflavones in red clover and subterranean clover. Chem. Ind. 1954 : 1019-1020.

Radford, H.M. and Wallace, A.L.C. (1974). C.N.S. blockade of the of the oestradiol-stimulated release of LH in the ewe. J. Endocrinol. 60 : 247-252.

Radford, H.M., Wallace, A.L. and Wheatley, I.S. (1970). LH release, ovulation and oestrus following the treatment of anoestrous ewes with ovarian steroids. J. Reprod. Fert. 21 : 371-372.

Radford, H.M., Wheatley, I.S. and Wallace, A.L.C. (1969). The effects of oestradiol benzoate and progesterone on secretion of luteinizing hormone in the ovariectomized ewe. J. Endocrinol. 44 : 135-136.

Ramirez, V.D. and Sawyer, C.H. (1974). A sex difference in the rat pituitary FSH response to unilateral gonadectomy as revealed in plasma radioimmunoassays. Endocrinol. 94: 475-482.

Reardon, T.F. and Robinson, T.J. (1961). Seasonal variation in the reactivity to oestrogen in the ovariectomized ewe. Aust. J. Agric. Res. 12: 320-326.

Reeves, J.J., Arimura, A. and Schally, A.V. (1971a). Pituitary responsiveness to purified luteinizing hormone-releasing hormone (LH-RH) at various stages of the oestrous cycle in sheep. J. Anim. Sci. 32: 123-126.

Reeves, J.J., Arimura, A. and Schally, A.V. (1971b). Changes in pituitary responsiveness to LH-RH in anoestrous ewes pre-treated with oestradiol benzoate. Biol. Reprod. 4: 88-92.

Reeves, J.J., O'Donnell, D.A. and Denorscia, F. (1972). Effect of ovariectomy on serum luteinizing hormone (LH) concentrations in the anoestrous ewe. J. Anim. Sci. 35: 73-78.

Reeves, J.J., Beck, T.W. and Nett, T.M. (1974). Serum FSH in the anoestrous ewe treated with 17β-oestradiol. J. Anim. Sci. 38: 374-377.

Restall, B.J. (1966). The fallopian tube of the sheep. II. The influence of progesterone and oestrogen on the secretary activities of the fallopian tube. Aust. J. Biol. Sci. 19: 187-197.

Rexroad, C.E. and Barb, C.R. (1977). Cervical mucus in oestrus ewes after treatment with oestrogen, progestagens and uterine devices. J. Anim. Sci. 44: 102-105.

Rexroad, C.E., and Casida, L.E. (1977). Effect of injection of progesterone into one ovary of PMSG-treated anoestrous ewe on follicle growth and ovarian oestradiol-17β. J. Anim. Sci. 44: 84-88.

Richards, J.S. and Midgley, A.R. (1976). Protein hormone action : A key to understanding ovarian follicular and luteal cell development. Biol. Reprod. 14 : 82-94.

Robison, G.A., Butcher, R.W. and Sutherland, E.W. (1971). from 'Cyclic AMP' Academic Press, New York.

Rodbard, D. (1968). Mechanics of ovulation. J. Clin. Endocrinol. Metab. 28 : 849-861.

Rondell, P. (1974). Role of steroid synthesis in the process of ovulation. Biol. Reprod. 10 : 199-215.

Rossiter, R.C. (1969). Physiological and ecological studies on the oestrogenic isoflavones in subterranean clover. VII. Effect of nitrogen supply. Aust. J. Agric. Res. 20 : 1043-1051.

Rossiter, R.C. (1970). Factors effecting the oestrogen content of subterranean clover pastures. Aust. Vet. J. 46 : 141-144.

Rossiter, R.C. and Barrow, N.J. (1972). Physiological and ecological studies on the oestrogenic isoflavones in subterranean clover. IX. Effects of sulphur supply. Aust. J. Agric. Res. 23 : 411-418.

Rossiter, R.C. and Beck, A.B. (1966a). Physiological and ecological studies on the oestrogenic isoflavone content of subterranean clover. I. Effects of temperature. Aust. J. Agric. Res. 17 : 29-37.

Rossiter, R.C. and Beck, A.B. (1966b). Physiological and ecological studies on the oestrogenic isoflavones in subterranean clover. II. Effects of phosphate supply. Aust. J. Agric. Res. 17 : 447-456.

Ruh, T.S. and Ruh, M.F. (1974). The effect of anti-oestrogens on the nuclear binding of the oestrogen receptor. Steroids 24 : 209-224.

Salamonsen, L.A., Jonas, H.A., Burger, H.G., Buckmaster, J.M., Chamley, W.A., Cumming, I.A., Findlay, J.K. and Goding, J.R. (1973). A heterologous radioimmunoassay for Follicle Stimulating Hormone; application to measurement of FSH in the ovine oestrous cycle and in several other species including man. Endocrinol. 93: 610-618.

Sanger, V.L. and Bell, M.S. (1959). Estrogenic activity in green forage crops and its effects on breeding ewes. J. Amer. Vet. Assn. 134: 237-239.

Scales, G.H. and Moss, R.A. (1976). Mating ewes on lucerne. N.Z.J. Agric. 132: 21-22.

Scales, G.H., Moss, R.A. and Kelly, R.W. (1977). Reproductive performance of ewes mated on lucerne. Proc. N.Z. Soc. Anim. Prod. 37: 152-157.

Scaramuzzi, R.J. (1975). Inhibition of oestrus behaviour in ewes by passive immunization against oestradiol-17β. J. Reprod. Fert. 42: 145-148.

Scaramuzzi, R.J., Lindsay, D.R. and Shelton, J.N. (1971a). The effect of oestradiol benzoate on the duration of oestrus behaviour in the ovariectomized ewe. J. Endocrinol 50: 345-346.

Scaramuzzi, R.J., Lindsay, D.R. and Shelton, J.N. (1972). Effect of repeated oestrogen administration on oestrus behaviour in ovariectomized ewes. J. Endocrinol. 52: 269-278.

Scaramuzzi, R.J., Tillson, S.A., Thorneycroft, I.H. and Caldwell, B.V. (1971b). Action of exogenous progesterone and estrogen on behavioural estrus and luteinizing hormone levels in the ovariectomized ewe. Endocrinol. 88: 1184-1189.

Schinckel, P.G. (1948). Infertility in ewes grazing subterranean clover pastures. Observations on breeding behaviour following transfer to 'sound' country. Aust. Vet. J. 24 : 289-294.

Schwartz, N.B. (1974). The role of FSH and LH and of their antibodies on follicle growth and on ovulation. Biol. Reprod. 10 : 236-272.

Seamark, R.F. (1978). Intraovarian factors in ovarian function. Proc. 6th Asia and Oceania Cong. Endocrinol : pp. 165-171.

Seamark, R.F., Moor, R.M. and McIntosh, J.E.A. (1974). Steroid hormone production by sheep ovarian follicles cultured In vitro. J. Reprod. Fert. 41 : 143-158.

Shemesh, M., Lindner, H.R. and Ayalon, N. (1972). Affinity of rabbit uterine oestradiol receptor for phyto-oestrogens and its use in a competitive protein binding R.I.A. for plasma coumestrol. J. Reprod. Fert. 29 : 1-9.

Shutt, D.A., Axelsen, A. and Lindner, H.R. (1967). Free and conjugated isoflavones in the plasma of sheep following ingestion of oestrogenic clover. Aust. J. Agric. Res. 18 : 647-655.

Shutt, D.A. and Braden, A.W.H. (1968). The significance of equol in relation to the oestrogenic responses in sheep ingesting clover with a high formononeton content. Aust. J. Agric. Res. 19 : 545-555.

Shutt, D.A., Braden, A.W.H. and Lindner, H.R. (1969). Plasma coumestrol levels in sheep following administration of synthetic coumestrol or ingestion of medic hay (Medicago littoralis). Aust. J. Agric. Res. 20 : 65-69.

Shutt, D.A., Weston, R.H. and Hogan, J.P. (1970). Quantitative aspects of phyto-oestrogen metabolism in sheep fed on subterranean clover (Trifolium subterranean) cultivar Clare or red clover (Trifolium pratense). Aust. J. Agric. Res. 21: 713-722.

Skarzynski, B. (1933). An oestrogenic substance from plant material. Nature 131 : 766.

Smeaton, T.C. and Robertson, H.A. (1971). Studies on the growth and atresia of Graafian follicles in the ovary of the sheep. J. Reprod. Fert. 25 : 243-252.

Smith, J.F. (1971). Studies on ovine infertility in agricultural regions of Western Australia : Cervical mucus production by fertile and infertile ewes. Aust. J. Agric. Res. 22 : 513-519.

Smith, J.F. (1975). Studies on ovine infertility in agricultural regions of Western Australia : Plasma progesterone levels of fertile and infertile ewes. Aust. J. Biol. Sci. 28 : 85-88.

Smith, J.F., Jagusch, K.T. and Brunswick, L. (1978). unpublished data. Ruakura Agric. Res. Centre. Hamilton New Zealand.

Smith, J.F. and Robinson, T.J. (1969). Luteal function in Merino ewes and the effect of exogenous progestagens. J. Endocrinol. 44 : 79-89.

Snedecor, G.W. and Cochrane, W.G. (1967). from 'Statistical methods' Iowa State University Press.

Squires, V.R. (1966). Seasonal incidence of oestrogenic activity in irrigated pasture as assessed by wether teat length increase. Proc. Aust. Soc. Anim. Prod. 6 : 117-122.

Steinberger, E. and Chowdhury, M. (1977). The effects of testosterone proprionate and estradiol benzoate on the In vitro synthesis of FSH. Biol. Reprod. 16 : 403-408.

Stob, M., Davis, R.L. and Andrews, F.N. (1957). Strain differences in the oestrogenicity of alfalfa. J. Anim. Sci. 16 : 850-853.

Stormshak, F., Kelly, H.E. and Hawk, H.W. (1969). Suppression of ovine luteal formation by oestradiol-17β. J. Anim. Sci. 29 : 476-478.

Stuthman, D.D., Bickoff, E.M., Davis, R.L. and Stob, M. (1967). Coumestrol differences in Medicago sativa L. free of foliar disease symptoms. Crop Sci. 6 : 333-334.

Sundaram, S.K. and Stob, M. (1967). Effect of unilateral ovariectomy on reproduction and induced ovulation in ewes. J. Anim. Sci. 26 : 374-376.

Sutherland, R.L. and Mester, J. (1976). Effect of oestradiol and progesterone on the concentration of oestrogen receptor sites in the chicken oviduct. J. Endocrinol. 71 : 55p.

Thimonier, J., Terqui, M. and Pelletier, J. (1974). Plasma estradiol and progesterone levels and pituitary responsiveness to LH-RH in the ewe. J. Steroid Biochem 5 : 388.

Tojo, S., Fukunishi, H., Tsuchihashi, T., Shimura, T., Mikami, K. and Kaku, H. (1975). Electrical activity recorded from ovarian tissue in perfused human utero-tubo-ovarian unit. J. Reprod. Fert. 44 : 587-589.

Trounson, A.O., Chamley, W.A., Kennedy, J.P. and Tassell, R. (1974). Primordial follicle numbers in ovaries and levels of LH and FSH in pituitaries and plasma of lambs selected for and against multiple births. Aust. J. Biol. Sci. 27 : 293-299.

Tsafriri, A., Lindner, H.A., Zor, U. and Lamprecht, S.A. (1972). Physiological role of prostaglandins in the induction of ovulation. Prostaglandins 2 : 1-10.

Turnbull, K.E., Braden, A.W.H. and George, J.M. (1966). Fertilization and early embryonic losses in ewes that had grazed oestrogenic pasture for 6 years. Aust. J. Agric. Res. 17 : 907-917.

Turnbull, K.E., Braden, A.W.H. and Mattner, P.E. (1977a). The pattern of follicular growth and atresia in the ovine ovary. Aust. J. Biol. Sci. 30: 229-241.

Turnbull, K.E., Land, R.B. and Scaramuzzi, R.J. (1977b). Patterns of growth of Graafian follicles in sheep and their relationship to ovulation rate. Proc. Aust. Soc. Reprod. Biol. 9: 45.

Turnbull, K.E., Shutt, D.A. and Braden, A.W.H. (1967). Increase in chlorine content of cervical mucus as a simple test for impending ovulation and for oestrogenic stimulation in ewes. Aust. J. Exptal. Anim. Husb. 7: 314-317.

Underwood, E. J. and Shier, F.L. (1951). The permanence of the oestrogenic effects of subterranean clover grazing on the ewe. Aust. Vet. J. 27: 63-67.

Wallace, L.R. (1961). Influence of liveweight and condition on ewe fertility. Proc. Ruakura Farmers Conf. p.14.

Walles, B., Edvinsson, L., Owman, C., Sjoberg, N.O. and Sporrong, B. (1976). Cholinergic nerves and receptors mediating contraction of the Graafian follicle. Biol. Reprod. 15: 565-572.

Walles, B., Falck, B., Owman, C. and Sjoberg, N. (1977). Characterization of autonomic receptors in the smooth musculature of human Graafian follicle. Biol. Reprod. 17: 423-431.

Weiss, T.J. and Armstrong, D.J. (1977). Adenylate cyclase activity in sheep ovarian follicular tissue; developmental changes in hormone response. Proc. Aust. Soc. Reprod. Biol. 9: 46.

Welchen, R. and Dullaart, J. (1976). Administration of anti-serum against ovine FSH or ovine LH at pro-oestrus in the rat; effects on follicular development during the oncoming cycle. J. Endocrinol. 70: 301-306.

Wheeler, A.G., Baird, D.T., Land, R.B. and Scaramuzzi, R.J. (1977a). Seasonal variation in oestrus, the secretion of oestrogen and progesterone and LH levels the ewe. J. Reprod. Fert. 51: 427-432.

Wheeler, A.G., Baird, D.T., Land, R.B. and Scaramuzzi, R.J. (1977b). Genetic variation in the secretion of oestrogen in the ewe. J. Endocrinol. 75: 337-338.

Wise, P.M. and Payne, A.H. (1975). The oestrogen cytosol receptor in female ovine pituitary. Molec. Cell. Endocrinol. 3: 385-395.

Wong, E. (1962). Detection and estimation of oestrogenic constituents in red clover. J. Sci. Food Agric. 13: 304.

Wong, E. and Flux, D.S. (1962). The oestrogenic activity of red clover isoflavones and some of their degradation products. J. Endocrinol. 24: 341-348.

Wong, E., Flux, D.S. and Latch, G.C.M. (1971). The oestrogenic activity of white clover. N.Z. J. Agric. Res. 14: 639-645.

Wong, E. and Latch, G.C.M. (1971). Effect of fungal diseases on phenolic contents of white clover. N.Z. J. Agric. Res. 14: 633-638.

Wong, M.S.F. and Cox, R.I. (1971). Evidence for phyto-oestrogens as sulpho-conjugates in sheep plasma. Proc. 4th Asia and Oceania Cong. Endocrinol. Absts. P.122.

Wong, M.S.F. and Cox, R.I.(1972). Microanalysis of phyto-oestrogens. J. Reprod. Fert. 28: 158-159.

Wroth, R.H. and Lightfoot, R.J. (1976). A study of reproductive wastage among commercial sheep flocks grazing oestrogenic pastures in south Western Australia. Proc. Aust. Soc. Anim. Prod. 11: 225-232.

Yang, N.S.T., Marsh, J.M. and Le Maire, W.J. (1974). Post ovulatory changes in the concentration of prostaglandins in rabbit Graffian follicles. Prostaglandins 6: 37-44.

Yuthasastrakosol, P., Howland, B.E., Simaraks, S. and Palmer, W.M. (1974). Oestrogen-induced LH release in progesterone-treated ovariectomized ewes. Can. J. Anim. Sci. 54: 565-572.